Preparation, characterization and antibacterial activity of biodegradable films prepared from carrageenan

G. El-Fawal

Revised: 15 December 2013 / Accepted: 26 December 2013 / Published online: 22 January 2014
© Association of Food Scientists & Technologists (India) 2014

Abstract Carrageenan films have been formulated as a packaging material. Films plasticized with glycerol were loaded with citric acid (1, 0.75, 0.5, 0.25 and 0.1 %) for enhanced antimicrobial effects. Blank and citric acid loaded films were characterized by mechanical properties, scanning electron microscopy and contact angle. In addition, swelling and antibacterial studies were conducted to further characterize the films. Both blank and citric acid loaded films showed different morphology, high elasticity and acceptable tensile (mechanical) properties. These citric acid loaded films produced higher zones of inhibition against *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Dickeya chrysanthemi* strains compared to blank film.

Keywords Carrageenan · Citric acid · Antibacterial · Packaging material

Introduction

Presently the industrial application of natural polymers and their derivatives is the most fascinating and vastly investigated field. For this purpose, biodegradable natural polymers such as polysaccharides and protein have been widely used (Younes and Cohn 1987; Jeong et al. 1997; Wijesinghe and You-Jin 2012; Archana et al. 2013; El-Aassar et al. 2013; Moustafa et al. 2013).

Carrageenan are high-molecular weight polysaccharides with three major fractions (k-kappa, i-iota and l-lambda),

which vary in the number and position of the sulphate groups on the galactose dimer. The k- and i-types form gels through the 3,6-anhydro-D-galactopyranosyl ring which can adopt the double helical structure necessary for the gelation process (Therkelsen 1993; Ribeiro et al. 2004).

Carrageenan and its derivatives form valuable ingredients for foods, cosmetics and pharmaceuticals (Ruiter and Rudolph 1997; Uruakpa and Arntfield 2006; Hadi and Ida Idayu 2013). In recent years, they have demonstrated to play significant role in antioxidant activities (Yuan et al. 2006; Zhang et al. 2003, 2004) and have been explored as effective excipients in controlled release drug delivery systems (Makino et al. 2001; Lazzarini et al. 2006; Joshua et al. 2013).

Kappa-carrageenan is mostly used in the food industry as gelling, stabilizing agent and thickener because of its high hydrophilicity, mechanical strength, biocompatibility and biodegradability (Guisseley et al. 1980). Kappa-carrageenan would reduce or eliminate toxicity in biomedical applications. For this reason, Kappa-carrageenan has been applied for immobilizing protein and controlled drug delivery systems (Palace et al. 1999; Muhamad et al. 2011; Hadi and Ida Idayu 2013).

Recent studies have focused on the application of biodegradable films on the food surface preventing the diffusion of preservative into the food and inhibiting surface microbial growth (Lian-Syun et al. 2009; İlke et al. 2011; Soliman et al. 2012).

Organic acids such as citric, succinic, lactic acids and succinic anhydride exhibit both bacteriocidal and bactiostatic effects against pathogenic strains (Bal'A and Marshall 1998; Mani-López et al. 2012). Citric acid was used to obtain natural antimicrobial packaging material.

The aim of this study is to prepare of flexible and bioactive films from Kappa-carrageenan and determined their antibacterial activity against gram-positive (*Staphylococcus aureus* (*S. aureus*), *Dickeya chrysanthemi* (*D. chrysanthemi*)) and

G. El-Fawal (✉)
Polymer Materials Research Department, Advanced Technology
and New Materials Research Institute, SRTA City,
New Boarg El-Arab City 21934, Alexandria, Egypt
e-mail: gelfawal@gmail.com

gram-negative bacteria (*Escherichia coli* (*E. coli*), *Proteus mirabilis* (*P. mirabilis*), *Pseudomonas aeruginosa* (*P. aeruginosa*)).

Materials and methods

Material

Kappa-carrageenan, citric acid and all the chemicals were obtained from Sigma-Aldrich. Distilled water was also used in film preparation. All chemicals were used as received with no additional purification.

Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213 and *Dickeya chrysanthemi* JF972567) and gram-negative bacteria (*Escherichia coli* ATCC 25922, *Proteus mirabilis* O10, S1959 (OJK, O3) and *Pseudomonas aeruginosa* ATCC 27853) were kindly supplied by Dr/ Elsayed Hafez (Genetic Engineering & Biotechnology Research Institute, SRTA-city).

Film preparation

Kappa -Carrageenan (KC) formulations for films were prepared by dissolving a definite amount of KC (2 g) in 100 ml of distilled water containing 25 % of glycerol (Gly) depending on KC weight (at 70 °C) with strong mixing in a helix mixer for 30 min. Afterwards, pre-determined amounts of citric acid (CA), were added to KC solution, so their concentrations were 1 (KC-1), 0.75(KC-2), 0.5 (KC-3) and 0.1 (KC-4) %, respectively, based on the weight of KC. Finally, samples were poured onto polystyrene Petri dishes and dried overnight at 40 °C to form film. Films were stored in desiccators till using.

Film thickness

Thicknesses of the films were measured with an electronic digital micrometer (Mitutoyo, Japan) which has a sensitivity of 0.001 mm and measurements were taken at least from five random positions on each film. Then, the mean value was used for the calculations of mechanical properties.

Tensile strength and elongation measurements

Tensile strength (TS) and elongation (E) of the prepared films were determined with an Shidmadzu universal testing machine (model AG-I 5 KN, Shidmadzu, Japan). Initial grip separation was set at 5 cm, and cross-head speed was set at 5 mm/min. TS was calculated by dividing maximum load (force) by initial cross-sectional area of a specimen. E was expressed as percentage of change of initial gauge length of a specimen (5 cm) at the point of sample failure.

Contact angle analysis

The contact angle means the inside angle between the surface of the KC film and the tangent to the surface of the water (Babak et al. 2007). It is well-known that the water contact angle will increase with increasing surface hydrophobicity. The surface hydrophobicity or wettability of the films was evaluated by contact angle measurements carried out with water using a goniometer (Ramé - hart, model 500-F1, France). To perform the measurements, a syringe was filled with 5 ml of water, and a drop was placed on top of the film surface that was glued on a well-leveled smooth platform. The angle between the baseline of the drop and the tangent at the drop boundary was measured. For each film type, at least three measurements on different positions of film surface were made and the average was taken.

Swelling studies

Swelling studies of films were carried out in water at room temperature. Samples were immersed in Petri dish filled with 30 ml of water solution. At different intervals, films were moved out, and the surface water of films was wiped using filter paper and weighed. Fresh water was used for further swelling study. The swelling percentage of films is then calculated:

$$\text{Swelling percentage(\%)} = \left[\frac{W_t - W_0}{W_0} \right] \times 100$$

Where W_t is the weight of swollen films at time t and W_0 is the initial weight of samples (Hadi and Ida Idayu 2013). Tests were conducted in triplicate to minimize error and were reported as a mean value.

Table 1 Influence of CA concentration on tensile strength (TS) and elongation (E) of KC films

Films	Mechanical properties	
	Tensile strength (TS)	Elongation (E)
KC-0	143.72±43.06 ^a	220.31±86.76 ^a
KC-1	17.67±8.27 ^c	19.0974±14.18 ^c
KC-2	19.57±3.37 ^c	24.4762±10.87 ^c
KC-3	33.31±7.48 ^c	21.7361±9.7 ^c
KC-4	49.12±23.31 ^c	30.3778±20.13 ^c
KC-5	93.43±26.47 ^b	105.69±58.9 ^b

$N=3$

Column values followed by the same letter are not significantly different ($P \leq 0.05$) based on Duncan's multiple range test. KC-0: K-carrageenan film without citric acid; KC-1, KC-2, KC-3, KC-4, and KC-5 are K-carrageenan film with 1, 0.75, 0.5, 0.25, 0.1 % citric acid concentration, respectively

Table 2 Influence of CA concentrations on contact angle of KC films

Films	Contact angle (θ)	
	Upper	Lower
KC-0	65.32 $\pm$ 4.61 ^a	72.59 $\pm$ 2.56 ^a
KC-1	21.34 $\pm$ 3.26 ^d	36.01 $\pm$ 3.44 ^d
KC-2	34.15 $\pm$ 175 ^c	44.04 $\pm$ 4.74 ^{cd}
KC-3	46.68 $\pm$ 4.93 ^b	47.76 $\pm$ 7.33 ^c
KC-4	62.56 $\pm$ 2.77 ^a	58.02 $\pm$ 3.23 ^b
KC-5	65.75 $\pm$ 8.6 ^a	63.16 $\pm$ 7.28 ^b

N=3

Column values followed by the same letter are not significantly different ($P \leq 0.05$) based on Duncan's multiple range test. KC-0: K-carrageenan film without citric acid; KC-1, KC-2, KC-3, KC-4, and KC-5 are K-carrageenan film with 1, 0.75, 0.5, 0.25, 0.1 % citric acid concentration, respectively

Microstructure examination

The surface of the films were analyzed using a scanning electron microscope (JEOL, model JSM-6460LV, Tokyo, Japan). For this purpose, the film pieces were mounted on bronze stubs using a double-sided tape and then coated with gold, before being observed under the microscope.

Antimicrobial activities

The antimicrobial activities of KC films were determined using the agar diffusion method (Ko et al. 2001; Sanjurjo et al. 2006; Soliman et al. 2012).

Bacteria culture and in vitro antibacterial assays In order to study the antimicrobial activity of the films, five pathogenic strains including two gram-positive bacteria (*S. aureus* and *D. chrysanthemi*) and three gram-negative bacteria (*E. coli*, *P. mirabilis* and *P. aeruginosa*) were used as test microorganism.

Bacteria culture All the strains were cultured at 37 °C for 24 h. By appropriately diluting with sterile solution, the cultures of bacteria containing 10⁷ CFU/mL were prepared and used for the antibacterial test.

Antimicrobial assay in vitro The agar diffusion method was used to analyze the antimicrobial effect of the prepared films on bacterial strains. Agar plates were inoculated with 100 μ l of bacterial cultures grown overnight (colony count 10⁷ CFU/ml). Film discs of 1 cm of diameter were placed on the inoculated agar plates. The plates were incubated at 37°C for 24 h. The diameter of the inhibition zone was measured with a caliper and indicated by cm depending on the diameter of the zone. Analyses were realized in triplicate.

Statistical planning of experiments

Statistics on a completely randomized design were performed with the analysis of variance (ANOVA) ($P < 0.05$) procedure in SPSS software (version 10.0 for windows, SPSS Inc., 233 South Wacker Drive, 11th Floor, Chicago, IL, USA).

Results and discussions

Tensile strength (TS) and elongation (E)

KC produces flexible, transparent and uniform films due to its viscoelastic nature (Alves et al. 2006; Campo et al. 2009; Joshua et al. 2013).

The mechanical properties of KC films prepared from unmodified and modified KC were shown in Table 1. KC-0 films exhibited high strength strength with high percentage of elongation. Whereas, the opposite mechanical behavior was noticed for modified KC films which showed lower tensile strength with lower elongation comparing with KC-0 films.

Films prepared using CA at various concentration show different trend for TS and E percentage. For example, KC-5 films had the better results for TS and E than the other films.

Table 3 Swelling percentage (%) of KC films

Time (min)	KC films/Swelling percentage (%)					
	KC-0	KC-1	KC-2	KC-3	KC-4	KC-5
5	559.6 $\pm$ 32.39 ^d	209.6 $\pm$ 6.2 ^a	180.43 $\pm$ 18.25 ^c	294.63 $\pm$ 22.85 ^b	291.97 $\pm$ 66.16 ^c	312.37 $\pm$ 9.89 ^c
10	692.2 $\pm$ 10.99 ^c	189.9 $\pm$ 11.4 ^a	236.63 $\pm$ 32 ^b	336.6 $\pm$ 32.33 ^b	351.83 $\pm$ 36.25 ^{bc}	476.5 $\pm$ 80.3 ^b
15	868.27 $\pm$ 15.14 ^b	228.37 $\pm$ 30.95 ^a	315.83 $\pm$ 32.42 ^a	316.8 $\pm$ 49.31 ^b	459.73 $\pm$ 99.6 ^b	581.05 $\pm$ 81.75 ^b
20	1015.89 $\pm$ 13.98 ^a	212.95 $\pm$ 23.55 ^a	326.27 $\pm$ 20.78 ^a	428.97 $\pm$ 61.96 ^a	601.65 $\pm$ 33.05 ^a	781.16 $\pm$ 47.47 ^a

N=3

Column values followed by the same letter are not significantly different ($P \leq 0.05$) based on Duncan's multiple range test. KC-0: K-carrageenan film without citric acid; KC-1, KC-2, KC-3, KC-4, and KC-5 are K-carrageenan film with 1, 0.75, 0.5, 0.25, 0.1 % citric acid concentration, respectively

Fig. 1 SEM images of the KC films showing **a** KC-0 films, **b** KC-1 and **c** KC-5

As TS and E were 93.43 ± 26.47 and 105.69 ± 58.9 , respectively. Also from the table it was clear that increasing CA concentration lead to decrease in TS and E. The best result was obtained using 0.1 % concentration and the bad result was obtained using 1 % concentration of CA.

Since Gly has multiple hydroxyl groups that penetrated into the polymeric network and formed hydrogen bonds with the hydroxyl groups of the polymers, this created enlarged spaces between the polymeric chains and resulted in increased flexibility of the films (Joshua et al. 2013). Gly also produced smooth and homogeneous films by reducing the crystallinity. Based on above, the blank KC-0 film was the better one. On the other hand, addition of different concentration of CA lead to change in the structure of final polymer as it has an additive plasticizing effect due to the presence of multiple hydroxyl groups. This lead to change in mechanical properties of films and this was confirmed by results in Table 1.

Contact angle

The main objective of this study was to change the properties of films by addition of cross-linker compound; therefore, the superficial hydrophilicity of films was determined by the contact angle method. In general, higher angles represent higher hydrophobic surface. When KC film is used for food packaging, choosing between the side with a high contact angle or a low contact angle should be considered (El Fawal 2013). So the

measurements were performed in both film sides: the upper one, which was in contact with air during the drying step and the lower one, which was in contact with the polystyrene Petri dish.

Table 2 showed the contact angles of KC films that indicate influence of CA concentrations on hydrophilicity of the prepared films.

These data exhibited that KC-0 film has a relatively high contact angle (Upper, $65.32 \pm 4.61^\circ$ and lower 72.59 ± 2.56). The contact angles for all prepared films using CA were lower than that value. Contact angles (upper and lower) of all films were decreased with increasing CA concentrations.

It was indicated from the aforementioned results that KC-0 films characterized with a relatively high contact angle because of nature of its structure. Whereas, forming KC films using CA decreased the hydrophobicity of the resultant films as a result of change the conformational structure of KC chains leading to exposing the more hydrophilic functional groups of CA.

Swelling

Results of swelling percentages (%) are illustrated in Table 3. The impact of time was more pronounced with extension. And so swelling percentages was higher at 20 min. for all films comparing with those at the other times, the only exception is offered by KC-1 at 15 min. The swelling percentage take the

Table 4 Antibacterial activity of KC films

Film	Bacteria/Growth inhibition zone (mm)				
	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>P. mirabilis</i>	<i>D. chrysanthemi</i>
KC-0	-ve	-ve	-ve	-ve	-ve
KC-1	4.18 ± 0.28^a	3.68 ± 0.17^a	3.55 ± 0.17^a	3.93 ± 0.09^a	3.25 ± 0.29^a
KC-2	3.25 ± 0.65^b	3.28 ± 0.19^a	3.33 ± 0.24^a	3.33 ± 0.24^b	3.05 ± 0.1^a
KC-3	3.38 ± 0.15^b	2.03 ± 0.13^b	2.15 ± 0.13^b	3.38 ± 0.95^b	3.25 ± 0.29^a
KC-4	2.83 ± 0.24^b	1.43 ± 0.36^c	-ve	2.4 ± 0.27^c	3.13 ± 0.96^a
KC-5	1.53 ± 0.96^c	-ve	-ve	-ve	-ve

N=3

Column values followed by the same letter are not significantly different ($P \leq 0.05$) based on Duncan's multiple range test. KC-0: K-carrageenan film without citric acid; KC-1, KC-2, KC-3, KC-4, and KC-5 are K-carrageenan film with 1, 0.75, 0.5, 0.25, 0.1 % citric acid concentration, respectively

sequence KC-0 (1015.89 ± 13.98 %) followed by KC-5 (781.16 ± 47.47) and the lowest percentage was for KC-1 (212.95 ± 23.55). On the other hand, swelling percentages for each film increased with increasing time. In addition swelling percentages decreased with increasing CA concentration (at fixed time), as swelling percentage sequence would be $KC-0 > KC-5 > KC-4 > KC-3 > KC-2 > KC-1$. Low swelling with increasing CA concentration can be due to the protonation of carboxylic groups and creation of more hydrogen bonds in KC hydroxyl groups resulting in more compact networks and therefore less swelling (Hadi and Ida Idayu 2013).

Microstructure

The surface microstructures depended on the drying conditions (evaporation rate of the solution) and especially depend on the humidity during drying (Tomoyuki et al. 2000). The upper film surface, which was in contact with air during the drying step, was used here.

The morphologies of KC films were shown in Fig. 1. In this study, different morphologies were observed in different samples. KC-0 film, Fig. 1a, show compact and dense structures with little cavities and voids were detected and continuous even matrix was not found, although presence of plasticizer increased association within polymer chains (Pereda et al. 2010).

On other hands, micrographs of KC-1 and KC-5 (Fig. 1b, c) exhibited that topography of these films had rougher surface than the KC-0 film as there were crystals present on the surface of films. However, number and distribution of crystals different based on the concentration of CA, where the KC-1 films characterized with more dense crystals structure comparing with the other film. These noticeable topographic features of films owing to the way which KC, CA and Gly interact with each other depending on CA concentrations and reaction conditions.

Antimicrobial effect

Recent studies have focused on the application of biodegradable films on the food surface preventing the diffusion of preservative into the food and inhibiting surface microbial growth (Lian-Syun et al. 2009; İlke et al. 2011; El Fawal 2013).

The antimicrobial properties of the films were evaluated by noting whether there was a clear inhibitory zone around the film or not after incubation. All strains were tested before using in this experiment with a reference antibiotic to be sure of efficiency of it (In labs of Genetic Engineering & Biotechnology Research Institute, SRTA-city).

The capabilities of all KC films in inhibiting the growth of the tested bacteria were assessed (Table 4). The results of the research revealed that the KC-0 film itself could not inhibit the microbial growth around the film (i.e. there is no inhibition zone).

The obtained results revealed that the KC-0 film have no antibacterial activity against these bacterial strains. Whereas, KC films containing CA exhibited higher antibacterial effect on the tested strains. This inhibitory effect was differed according to concentration of CA and type of tested bacterial strains. KC-1 film exhibited higher inhibitory effect on all stains comparing with the other films. This refers to high concentration of CA in this film.

On the other side, the inhibitory effect for each strain decreased with decreasing in CA concentration. For example inhibition zone for *E. coli* decreased from 3.68 ± 0.17 cm using KC-1 film to 1.43 ± 0.36 cm using KC-4 film and inhibition zone became zero using KC-5 film, which had the low concentration of CA.

KC-5 had not effect on all strains except *P. aeruginosa* due to low concentration of CA which could not suppress bacterial growth.

The antibacterial action of these films arises from functional groups of acid that affect the electrostatic interactions between these species and cell membrane. Besides, the decided structural properties of the KC films containing CA films can affect on the adhesion of bacterial cells and consequently on their ability for inhibition of bacterial growth (Soliman et al. 2012).

Conclusions

KC films were prepared, mechanical, wettability, contact angle properties and their antibacterial activity against, *E. coli*, *P. aeruginosa*, *P. mirabilis*, *D. chrysanthemi* and *S. aureus* was assessed. The results exhibited that this technique had a potential for modify the properties of KC films such as mechanical, solubility and antibacterial activity. Preparation of KC films may hold practical significance in changing the flexibility, solubility and antibacterial activity. Therefore, these changes can extend the potential applications of KC films in packaging and biomedical fields.

References

- Alves V, Costa N, Hilliou L, Larotonda F, Goncalves M, Sereno A (2006) Design of biodegradable composite films for food packaging. *Desalination* 199:331–333
- Archana G, Sabina K, Babuskin S, Radhakrishnan K, Mohammed A, Azhagu P, Sivarajan M, Sukumar M (2013) Preparation and characterization of mucilage polysaccharide for biomedical applications. *Carbohydr Polym* 98:89–94
- Babak G, Mohamad M, Oromiehie AR, Keramat R, Elhame RR, Jafar M (2007) Effect of plasticizing sugars on water vapor permeability, surface energy and microstructure properties of zein films. *LWT* 40: 1191–1197
- Bal'A, Marshall DL (1998) Organic acid dipping of catfish fillets: effect on color, microbial load and *Listeria monocytogenes*. *J Food Protect* 11:1470–1474

- Campo VL, Kawano DF, Da Silva DB, Carvalho I (2009) Carrageenans: biological properties, chemical modifications and structural analysis—a review. *Carbohydr Polym* 77:167–180
- El Fawal G (2013) Thesis: protein-based films and coatings: use of natural antimicrobial agents for food preservation. Chemistry department, Faculty of science, Zagazig University, Egypt
- El-Aassar MR, Elsayed EH, Moustafa MG, Al-Deyab SS (2013) Synthesis, characterization, and antimicrobial activity of poly(acrylonitrile-co-methyl methacrylate) with silver nanoparticles. *Appl Biochem Biotechnol* 171:643–654
- Guisseley KB, Stanley NF, Whitehous PA (1980) Carrageenan in handbook of watersoluble gums and resins. McGraw-hill, New York
- Hadi H, Ida Idayu M (2013) Modification and swelling kinetic study of kappa-carrageenan-based hydrogel for controlled release study. *J Chin Inst Chem Eng* 44:182–191
- İlke UÜ, Figen K, Ahmet Y (2011) Active packaging of ground beef patties by edible zein films incorporated with partially purified lysozyme and Na₂EDTA. *J Food Sci Technol* 46:1289–1295
- Jeong B, Bae YH, Lee DS, Kim SW (1997) Biodegradable block copolymers as injectable drug-delivery systems. *Nature* 388:860–862
- Joshua SB, Harshavardhan VP, John T (2013) Polyox and carrageenan based composite film dressing containing anti-microbial and anti-inflammatory drugs for effective wound healing. *Int J Pharm* 441:181–191
- Ko S, Janes ME, Hettiarachchy NS, Johnson MG (2001) Physical and chemical properties of edible films containing nisin and their action against *Listeria Monocytogenes*. *J Food Sci* 66:1006–1011
- Lazzarini R, Maiorka PC, Liu J, Papadopoulos V, Palermo-Neto J (2006) Diazepam effects on carrageenan-induced inflammatory paw edema in rats: role of nitric oxide. *Life Sci* 78:3027–3034
- Lian-Syun L, Be-Jen W, Yih-Ming W (2009) Preservation of commercial fish ball quality with edible antioxidant-incorporated zein coatings. *J Food Process Preserv* 33:605–617
- Makino K, Idenuma R, Murakami T, Ohshima H (2001) Design of a rate- and time-programming drug release device using a hydrogel: pulsatile drug release from j-carrageenan hydrogel device by surface erosion of the hydrogel. *Colloids Surf B: Biointerfaces* 20:355
- Mani-López E, García HS, López-Malo A (2012) Organic acids as antimicrobials to control *Salmonella* in meat and poultry products. *Food Res Int* 45:713–721
- Moustafa MG, Fouda MR, El-Aassar, Al-Deyab SS (2013) Antimicrobial activity of carboxymethyl chitosan/polyethylene oxide nanofibers embedded silver nanoparticles. *Carbohydr Polym* 92:1012–1017
- Muhamad II, Fen LS, Hui HN, Mustapha NA (2011) Genipin-cross-linked kappa carrageenan/carboxymethyl cellulose beads and effects on beta-carotene release. *Carbohydr Polym* 83:1207–1212
- Palace GP, Fitzpatrick R, Tran KV, Phoebe HC, Norton K (1999) Determination of amino acids in diverse polymeric matrices using HPLC, with emphasis on agars and agaroses. *Biochim Biophys Acta* 509:1472
- Pereda M, Aranguren M, Marcovich N (2010) Caseinate films modified with Tung oil. *Food Hydrocoll* 24:800–808
- Ribeiro KO, Rodrigue MI, Sabadini SE, Cunha RL (2004) Mechanical properties of acid sodium caseinate-k-carrageenan gels: effect of cosolute addition. *Food Hydrocoll* 18:71–79
- Ruiter GA, Rudolph B (1997) Carrageenan biotechnology. *Trends Food Sci Technol* 8:389
- Sanjurjo K, Silvia FS, Gerschenson L, Jagus R (2006) Study of the performance of nisin supported in edible films. *Food Res Int* 39:749–754
- Soliman EA, Khalil AA, Deraz SF, El-Fawal G, Abd Elrahman S (2012) Synthesis, characterization and antibacterial activity of biodegradable films prepared from Schiff bases of zein. *J Food Sci Technol*. doi:10.1007/s13197-012-0792-y
- Therkelsen GH (1993) Carrageenan. In: Whistler RL, BeMiller JN (eds) *Industrial gums: polysaccharides and their derivatives*. Academic, California, pp 145–180
- Tomoyuki Y, Seiichiro I, Takaaki M (2000) Physical evaluation of pure zein films by atomic force microscopy and thermal mechanical analysis. *J Am Oil Chem Soc* 77(7):699–704
- Uruakpa FO, Amtfield SD (2006) Network formation of canola protein-j-carrageenan mixtures as affected by salts, urea and dithiothreitol. *LWT J Food Sci Technol* 39:939–946
- Wijesinghe W, You-Jin J (2012) Biological activities and potential industrial applications of fucose rich sulfated polysaccharides and fucoidans isolated from brown seaweeds: a review. *Carbohydr Polym* 88:13–20
- Younes H, Cohn D (1987) Morphological study of biodegradable PEO/PLA block copolymers. *J Biomed Mater Res* 21:1301–1316
- Yuan H, Song J, Zhang W, Li X, Li N, Gao X (2006) Antioxidant activity and cytoprotective effect of j-carrageenan oligosaccharides and their different derivatives. *Bioorg Med Chem Lett* 16:1329–1334
- Zhang QB, Yu PZ, Li ZE, Zhang H, Xu ZH, Li PC (2003) Antioxidant activities of sulfated polysaccharide fractions from *Porphyra haitanensis*. *J Appl Phycol* 15:305
- Zhang QB, Li N, Liu XG, Zhao ZQ, Li ZE, Xu ZH (2004) The structure of a sulfated galactan from *Porphyra haitanensis* and its in vivo antioxidant activity. *Carbohydr Res* 339:105–111