pISSN 1229-1153 / eISSN 2465-9223 *J. Food Hyg. Saf.*
Vol. 32, No. 5, pp. 443~446 (2017)
https://doi.org/10.13103/JFHS.2017.32.5.443

Journal of Food Hygiene and Safety
Available online at http://www.foodhygiene.or.kr

Polymer Hydrogels Formulated with Various Cross-Linkers for Food-Surface Application to Control *Listeria monocytogenes*

Sejeong Kim[1,2], Hyemin Oh[1,2], Heeyoung Lee[1,2], Soomin Lee[1,2], Jimyeong Ha[1,2],
Jeeyeon Lee[1,2], Yukyoung Choi[1,2], and Yohan Yoon[1,2]*
[1]*Risk Analysis Research Center, Sookmyung Women's University, Seoul, Korea*
[2]*Department of Food and Nutrition, Sookmyung Women's University, Seoul, Korea*

(Received September 12, 2017/Revised September 29, 2017/Accepted October 17, 2017)

ABSTRACT - This study investigated the physical properties of polymers and antimicrobial activities of organic acids on *Listeria monocytogenes* to develop hydrogels. κ-carrageenan (1, 2, and 3%), carboxymethylcellulose (CMC; 1, 3, and 5%), and agar (1.5 and 3%) were mixed with cross-linkers (Na^+, K^+, Ca^{2+}, and Al^{3+}) or each other by stirring or heating to form cross-linkage, and their physical properties (hardness, elasticity, and swelling) were measured. The hydrogels formulated with organic acid (1, 3, and 5%) were analyzed by spot assay against *L. monocytogenes*. κ-carrageenan formed hydrogels with high hardness without other cross-linkers, but they had low elasticity. The elasticity was improved by mixing with other cross-linkers such as K^+ or other polymer, especially in 3% κ-carrageenan. CMC hydrogel was formed by adding cross-linkers Al^{3+}, Na^+, or Ca^{2+}, especially in 5% CMC. Thus, stickiness and swelling for selected hydrogel formulations (two of κ-carrageenan hydrogels and three of CMC hydrogels) were measured. Among the selected hydrogels, most of them showed appropriate hardness, but only 3% κ-carrageenan-contained hydrogels maintained their shapes from swelling. Hence, 3% κ-carrageenan+0.2% KCl and 3% κ-carrageenan+1% alginate+0.2% KCl+0.2% $CaCl_2$ were selected to be formulated with lactic acid, and showed antilisterial activity. These results indicate that 3% κ-carrageenan hydrogels formulated with lactic acid can be used to control *L. monocytogenes* on food surface.

Key words: Polymer, Hydrogel, Carrageenan, CarboxyMethylCellulose (CMC)

Introduction

Listeria monocytogenes is foodborne illness bacterium, causing abortion, still birth, meningitis, and sepsis[1)]. The mortality rate of *L. monocytogenes* infection is about 20-30%[2)], and the pathogen is usually isolated from food surfaces[3)]. Thus, various technologies to inhibit *L. monocytogenes* have been developed, but the technology for non-thermal application is not very common.

Hydrogel is three-dimensional structure constructed from cross-linking between one or more polymers[4)]. The polymeric materials were hydrophilic, and thus, they can absorb water from environments and swell, but they are not dissolved in water due to their cross-linked network[5)]. Because of this property, hydrogels were developed in various fields such as wound healing, drug delivery, tissue engineering, and cosmetics which apply hydrogels as vehicle or reservoirs for functional materials[6-8)]. The hydrogel has different mechanical properties determined by polymer, cross-linker, or gelling conditions[9)].

For applying hydrogels on food surface, natural materials are appropriate. κ-carrageenan, carboxymethylcellulose (CMC), agar, etc. should be appropriate for making hydrogels due to their nontoxicity, biodegradability, and renewability[10)]. Among various carrageenans (κ-, ι-, and λ-carrageenan), κ-carrageenan forms strong and rigid gels in the presence of potassium ions, and it is a common hydrophilic polysaccharide found in various seaweeds[11,12)]. κ-carrageenan gels are formed when the polymers are heated and cooled, and the cooling rate affects mechanical properties of the gels[13)]. CMC is another natural polysaccharide gelling in the presence of cation such as Na^+, Ca^{2+}, and Al^{3+}[14)]. Agar is also a natural polymer. Heating and subsequent cooling can induce gelation of the agar[15)].

If hydrogels are functionalized by adding antimicrobial agents or antioxidants, the hydrogels can be non-thermal decontamination technology. Therefore, in this study the physical properties of the hydrogels produced from various

---

*Correspondence to: Yohan Yoon, Food microbiology lab., Dept. Food and Nutrition, Sookmyung Women's University. 100, 47-gil, Cheongpa-ro, Youngsan-gu, Seoul 04310, Korea
Tel: 82-2-2077-7585, Fax: 82-2-2077-7585
E-mail: yyoon@sookmyung.ac.kr

polymers and antilisterial activities of the hydrogels formulated with organic acids were investigated to develop an antilisterial hydrogel.

Materials and Methods

Preparation of hydrogels

κ-carrageenan was suspended in 6 mL of glycerol at 1, 2 and 3%, and distilled water (19 mL) with or without 0.2% KCl and $CaCl_2$ was added to the mixtures to provide Ca^+. After heating at 95°C for 10 min, the mixtures were poured onto petridish, and they were cooled at room temperature to form hydrogel16). CMC hydrogels were prepared according to a study by Lee et al. 17) and Roy et al.18). Briefly, CMC (1, 3, and 5%) and 3% alginate in glycerol were mixed with 0.2% tartaric acid (TTA)+dihydroxyaluminum aminoacetate (DDA) solution, 0.2% of $CaCl_2$, or 0.2% NaCl to provide Al^+, Ca^+, or Na^+, respectively, and stirred at room temperature until gels were formed. The gels were poured onto petridish, and followed by incubation at 42°C to dry the gels. To prepare agar hydrogels, agar powder (1.5 g or 3 g) was dissolved in 25 mL distilled water or glycerol to be mixed with 3% CMC and 3% CMC+0.2% (DAA+TTA), and the mixture was heated using microwave for 30 sec. The heated solution was poured onto petridish and cooled at room temperature for the gelation19).

Measurement of hydrogel mechanical properties

Hydrogels were cut into 2 cm × 2 cm, and the hydrogel pieces were analyzed by TA *Express* (Texture analyzer, Stable Micro Systems LTD., Surrey, UK) and hardness, elasticity and stickiness of hydrogels were calculated by Exponent *Lite Express* (Software, Stable Micro Systems LTD., Surrey, UK). For swelling measurement, 1 cm × 1 cm of the hydrogel pieces were prepared and weighed before and after placing the gels in 24-well plate containing 0.5 mL of distilled water for 24 h. Swelling conditions were determined by the weight changes of the gels.

Spot assay of antimicrobials

L. monocytogenes 1/2b, isolated from beef carcass in previous study20), was used in this study. The bacterial strain in tryptic soy broth (TSB; Becton, Dickinson and Company, Sparks, MD, USA) was subcultured in 14 mL TSB until the growth reached at log phase (OD_{600} = 0.7-1.0), and the culture was mixed with TSB+0.8% agar to be OD_{600} = 0.1. Three milliliter aliquots of the mixtures were poured on TSA, and left at room temperature until solidified. To prepare antilisterial hydrogels, the hydrogels (1 cm × 1 cm) were placed in 10 mL of 1, 3, and 5% lactic acid for 1 h, and the hydrogels were placed on the agar containing *L. monocytogenes*. After 24-h incubation at 30°C, the sizes (mm) of the clear zones were measured.

Statistical analysis

The study for antimicrobial activity of hydrogels was repeated three times. The data for the size of clear zones measuring antimicrobial activity of hydrogels were analyzed with the GLM procedure of SAS (version 9.2, SAS Institute, Cary, NC, USA). LS means among fixed effects were compared by pairwise *t*-test at alpha = 0.05.

Results and Discussion

In this study, hydrogels composed of natural polymer and its proper cross-linkers were prepared, and the physical properties of the hydrogels such as hardness, elasticity, stickiness and swelling were compared.

Hardness of κ-carrageenan hydrogel increased as concentration of κ-carrageenan increased (Table 1). Addition of K^+ into κ-carrageenan by adding 0.2% KCl increased the hardness of the hydrogel, but addition of 1% alginate did not increase the hardness of κ-carrageenan hydrogel (Table 1). Hydrogel was not formed by CMC itself (Table 1).

Table 1. Hardness of κ-carrageenan, carboxymethyl cellulose (CMC) and agar hydrogels formulated with various cross-linkers

Cross-linker	κ-carrageenan		
	1%	2%	3%
-	3.293	15.85	20.30
0.2% KCl	2.93	19.08	53.35
0.2% KCl + 0.2% $CaCl_2$	2.22	10.57	1.57
1% alginate	1.40	4.95	14.89
1% alginate + 0.2% KCl	1.41	8.53	6.35
1% alginate + 0.2% KCl + 0.2% $CaCl_2$	2.87	14.49	25.26
	CMC		
	1%	3%	5%
-	NF1)	NF	NF
0.2% (dihydroxyaluminum aminoacetate (DAA) + tartaric acid (TTA))	NF	64.30	226.63
3% alginate	NF	134.52	52.42
3% alginate + 0.2% NaCl	NF	2.94	119.07
3% alginate + 0.2% $CaCl_2$	NF	218.77	23.75
3% alginate + 0.2% (DAA+TTA)	NF	90.81	8.96
		Agar	
		1.5%	3%
3% CMC		6.16	16.62
3% CMC + 0.2% (DAA+TTA)		38.21	6.30

1)No hydrogel formation

Table 2. Elasticity of κ-carrageenan, carboxymethyl cellulose (CMC) and agar hydrogels formulated with various cross-linkers

	κ-carrageenan		
Cross-linker	1%	2%	3%
-	−0.016	−0.081	−0.044
0.2% KCl	−0.015	−0.1	−0.196
0.2% KCl + 0.2% CaCl$_2$	−0.037	−0.118	−0.015
1% alginate	−0.107	−0.093	−7.26
1% alginate + 0.2% KCl	−0.052	−0.27	−2.132
1% alginate + 0.2% KCl + 0.2% CaCl$_2$	−0.03	−0.218	−0.122
	CMC		
	1%	3%	5%
-	NF[1)]	NF	NF
0.2% (dihydroxyaluminum aminoacetate (DAA) + tartaric acid (TTA))	NF	−3.505	−0.507
3% alginate	NF	−0.7	−0.363
3% alginate + 0.2% NaCl	NF	−0.007	−0.463
3% alginate + 0.2% CaCl$_2$	NF	−3.594	−0.215
3% alginate + 0.2% (DAA+TTA)	NF	−0.848	−0.577
		Agar	
		1.5%	3%
3% CMC		−0.167	−0.3
3% CMC + 0.2% (DAA+TTA)		−0.777	−0.152

[1)] No hydrogel formation

Table 3. Stickiness and swelling of hydrogels, selected according to hardness and elasticity of hydrogels

Hydrogels	Stickiness	Swelling
3% κ-carrageenan + 0.2% KCl	−0.904	+[1)]
3% κ-carrageenan + 1% alginate + 0.2% KCl + 0.2% CaCl$_2$	−1.939	++[2)]
5% carboxymethyl cellulose (CMC) + 0.2% (dihydroxyaluminum aminoacetate (DDA) + tartaric acid (TTA))	−3.546	-[3)]
5% CMC + 3% alginate	−4.344	-
5% CMC + 3% alginate + 0.2% NaCl	−1.688	-

[1)] gel weight was changed under 10%.

[2)] gel weight was changed over 10% and shape was maintained.

[3)] gel was melted thoroughly.

However, CMC was cross-linked with cationic ions such as Na$^+$, Ca^{2+}, and Al^{3+} (Table 1). Especially, addition of 0.2% DAA+TTA for generating Al^{3+} into CMC constructed CMC hydrogels that had higher hardness than other cross-linkers (Table 1). Agar hydrogels could be simply formed by heat treatment, and the hardness of gels increased by adding CMC 3%+2% (DAA+TTA) (Table 1). Although hydrogels exhibit high hardness, if the elasticity of the hydrogels are not sufficiently high, hydrogels may be broken while they are applied. Hence, the elasticity of the hydrogels was further measured.

Elasticity values of hydrogels made from various polymer and cross-linker matrixes are presented in Table 2. As the value is close to 0, the elasticity of the gel is higher. In general, the elasticity of κ-carrageenan hydrogels was much higher than those of CMC and agar hydrogels. The elasticity values of κ-carrageenan hydrogels generally decreased as cross-linkers were added. Although the elasticity decreased by adding cross-linkers, the combination of 3% κ-carrageenan with 0.2% KCl that had the highest hardness exhibited comparatively higher elasticity than other combinations, and the combination of 3% κ-carrageenan with 1% alginate+0.2% KCl+0.2% CaCl$_2$ that had the second highest hardness also showed comparatively higher elasticity than other combinations (Table 2). As described above, CMC did not form hydrogel itself, and thus, adding cross-linkers into CMC were necessary. Among CMC hydrogels, a combination of 3% CMC with 3% alginate+0.2% NaCl had the most elastic properties, but it had low hardness. Among 5% CMC, combinations of 5% CMC with 0.2% (DDA+TTA), 3% alginate, and 3% alginate+0.2% NaCl had high hardness, and their elasticity values were −0.507, −0.363, and −0.464, respectively. Taken together of hardness and elasticity, 3% κ-carrageenan with 0.2% KCl, 3% κ-carrageenan with 1% alginate+0.2% KCl+0.2% CaCl$_2$, 5% CMC+0.2% (DDA+TTA), 5% CMC+3% alginate, and 5% CMC+3% alginate+0.2% NaCl seemed appropriate hydrogels. Thus, they were further-analyzed for stickiness. Most elasticity values of agar hydrogels were further from 0. Hence, agar hydrogels were not considered for further analysis.

As stickiness value is close to 0, the stickiness of hydrogel

Table 4. The sizes (mm; mean ± S.D) of clear zones formed by κ-carrageenan hydrogels formulated with lactic acid against *Listeria monocytogenes*

Hydrogels	Lactic acid			
	0%	1%	2%	3%
3% κ-carrageenan + 0.2% KCl	0^a	10 ± 1.5^b	19 ± 1.2^c	22 ± 2.0^d
3% κ-carrageenan + 1% alginate + 0.2% KCl + 0.2% CaCl$_2$	0^A	11 ± 1.2^B	19 ± 1.2^C	21 ± 1.2^D

$^{a-d, A-D}$means with different letters are significantly different ($p < 0.05$).

is low. Stickiness of 5% CMC-contained hydrogels was higher than 3% κ-carrageenan-contained hydrogels (Table 3). However, the 5% CMC-contained hydrogels were melted thoroughly during swelling test (Table 3). Hence, only 3% κ-carrageenan+0.2% KCl and 3% κ-carrageenan+1% alginate+0.2% KCl+0.2% $CaCl_2$ were used to contain antimicrobial and examined for antimicrobial activity of the hydrogels. Both κ-carrageenan hydrogels showed increased antilisterial activity as lactic acid concentration increased (Table 4).

In conclusion, κ-carrageenan can be used for hydrogel, especially at 3%, and the physical properties were improved by addition of 0.2% KCl or 1% alginate+0.2% KCl+0.2% $CaCl_2$. In addition, formulating lactic acid into the hydrogels showed antilisterial activity, which may suggest other organic acids to be formulated into hydrogels. Therefore, the 3% κ-carrageenan hydrogels formulated with lactic acid can be used to control *L. monocytogenes* on food surface.

Acknowledgment

This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ0119932016)" Rural Development Administration, Republic of Korea.

Disclosure Statement

No competing financial interests exist.

References

1. Swaminathan B., Gerner-Smidt P.: The epidemiology of human listeriosis. *Microb. Infect*., **9**, 1236-1243 (2007).
2. Ramaswamy V., Cresence V.M., Rejitha J.S., Lekshmi M.U., Dharsana K.S., Prasad S.P., Vijila H.M.: *Listeria*-review of epidemiology and pathogenesis. *J. Microbiol. Immunol. Infect*., **40**, 4-13 (2007).
3. Cole M. B., Jones M. V., Holyoak C.: The effect of pH, salt concentration and temperature on the survival and growth of *Listeria monocytogenes*. *J. Appl. Bacteriol*., **69**, 63-72 (1990).
4. Ahmed E.M.: Hydrogel: Preparation, characterization, and applications: A review. *J. Adv. Res.,* **6**, 105-121 (2015).
5. Rosiak J.M., Yoshii F.: Hydrogels and their medical applications. *Nucl. Instr. Meth. Phys. Res. B*, **141**, 56-64 (1999).
6. Kim S.J., Hahn S.K., Kim D.H., Lee Y.P.: Development of a novel sustained release formulation of recombinant human growth hormone using sodium hyaluronate microparticles. *J. Control. Release*., **104**, 323-335 (2005).
7. Zhou H.Y., Chen X.G., Kong M., Liu C.S., Cha D.S., Kennedy J.F.: Effect of molecular weight and degree of chitosan deacetylation on the preparation and characteristics of chitosan thermosensitive hydrogel as a delivery system. *Carbohydr. Polym.*, **73**, 265-273 (2008).
8. Trksak R.M., Ford P.J.: Sago-based gelling starches. *Vol. 7,422,638* (ed. U. S. Patent), National Starch and Chemical Investment Holding Corporation, New Castle, DE, USA (2008).
9. Gulrez S.KH., Al-Assaf S., Phillips G.O.: Hydrogels: methods of preparation, characterisation and application. In progress in molecular and environmental bioengineering from analysis and modeling to technology applications (Angelo Carpi Ed.) In Tech, Croatia, Europe (2011).
10. Pourjavadi A., Soleyman R., Bardajee G.R., Ghavami S.: Novel superabsorbent hydrogel based on natural hybrid backbone: optimized synthesis and its swelling behavior. *Bull. Korean Chem. Soc.,* **30**, 2680-2686 (2009).
11. Rhein-Knudsen N., Ale M.T., Meyer A.S.: Seaweed hydrocolloid production: an update on enzyme assisted extraction and modification technologies. *Mar. Drugs*, **13**, 3340-3359 (2015).
12. Kirk R.E., Othmer D.F.: Encyclopedia of chemical technology. (Kroschwitz J.I., Howe-Grant M., Eds.) Wiley, New York, U. S. A., Vol. 4, p. 942 (1992).
13. Iijima M., Hatakeyama T., Takahashi M., Hatakeyama H.: Effect of thermal history on kappa-carrageenan hydrogelation by differential scanning calorimetry. *Thermochim. Acta*., **452**, 53-58 (2007).
14. Pourjavadi A., Barzegar Sh., Mahdavinia G.R.: MBA-cross-linked Na-Alg/CMC as a samart full-polysaccharide superabsorbent hydrogels. *Carbohydr. Polym.,* **66**, 386-395 (2006).
15. Othman R., Yahaya A.H., Arof A.K.: A zinc-air cell employing a porous zinc electrode fabricated from zinc-graphite-natural biodegradable polymer paste. *J. Appl. Electrochem.,* **32**, 1347-1353 (2002).
16. Mohamadnia Z., Zohuriaan-Mehr M.J., Kabiri K., Jamshidi A., Mobedi H.: Ionically cross-linked carrageenan-alginate hydrogel beads. *J. Biomater. Sci. Polym. Ed*., **19**, 47-59 (2008).
17. Lee T.W., Kim J.C., Hwang S.J.: Hydrogel patches containing Triclosan for acne treatment. *Eur. J. Pharm. Biopharm.*, **56**, 407-412 (2003).
18. Roy N., Saha N., Kitano T., Saha P.: Biodegradation of PVP-CMC hydrogel film: A useful food packaging material. *Carbohydr. Polym.*, **89**, 346-353 (2012).
19. Meena R., Chhatbar M., Prasad K., Siddhanta A.K.: Development of a robust hydrogel system based on agar and sodium alginate blend. *Polym. Int*., **57**, 329-336 (2008).
20. Animal and Plant Quarantine Agency.: Prevalence of *Listeria monocytogenes* from farm and fresh meat at slaughterhouse and epidemiological analysis by molecular method.