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Textural and rheological properties of hydrolyzed Konjac Glucomannan and Kappa-Carrageenan: Effect of molecular weight, total content, pH and temperature on the mixed system gels

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Abstract

Rheological and textural properties of mixed gels of hydrolyzed Konjac glucomannan (KGM) and kappa-carrageenan (kC) were studied. The objective of the work was to investigate the effect of ratio, total content, molecular weight, pH and temperature of KGM on the mixed system gels. At 40% KGM the gel showed better hardness; whereas, the total content (1.0%-2.0% (w/w)) demonstrated that when it's higher, the hardness increased significantly (P<0.05) and lowers the springiness. In addition, hardness values were relatively higher than would be expected for hydrolyzed KGM in comparison with native KGM and kC mixed system gels. It was found out that the hydrolyzed KGM (LM-2) exhibited better texture properties and suitable for the mixt system gels KGM/kC. The influence of pH shift on the hardness was stabilized after the pH5. The rheological properties, showed the significant temperature effect difference between kC alone and kC-LM-2 with 34±0.2oC and 42±0.2°C, respectively, where the gels' moduli G' and G" meet at frequency 10 rad/s and 1% strain. Although, KGM and kC mixed system gels behaviors are said to be influenced by the choice β-mannanase in the hydrolysis and preparation mechanisms.

Key words: Hydrolyzed KGM, kC, Mixed system gel, Texture, Rheological properties

Introduction

Konjac gum is a hydrocolloid gum derived from the tubers of *Araceae amorphophallus*, which comprises 60–70% konjac glucomannan. It has been recognized as GRAS (generally recognized as safe) by a consensus of scientific opinion since 1994 (Khanna and Tester, 2006; Case et al., 1992). It is widely utilised in areas of the food industry, chemical engineering and petroleum drilling (Al-Ghazzewi et al., 2007). KGM has been utilized as a raw material in the food and polymer industries in China since ancient times (He et al., 2011). KGM is regarded as a non-caloric food and in its traditional form is a source of indigestible high quality dietary fiber (Pan et al., 2011). However, health Canada advisory released a counter-indication stating that natural health products containing the ingredient glucomannan in tablet, capsule or powder form, which are currently on the Canadian market, have a potential for harm if taken without at least 8 ounces of water or other fluid (Health Canada Advisory, 2010). As a result of its high viscosity, konjac gum is generally added to other hydrocolloids to increase the viscosity of blended systems and improve food quality (Yaseen et al., 2005). KGM can also be used for modification of carbohydrate in diabetes metabolism and also used in the preparation of composite materials, edible film, coating/packaging film, biodegradable film and controlled release matrix (Gao and Nishinari, 2004; Seleiman et al., 2010).

Polysaccharide, such as carrageenans are extracted from Gigartina, Chondrus, Iridaea and Eucheuma species. carrageenans can exist in forms of κ-, λ- and ι-; among which κ-carrageenan is characterised by its repeating disaccharide units of 3-linked-β-D-galactose-4-sulfate and 4-linked-3,6 anhydro-α-D-galactose. κ-carrageenan have the ability to form a gel in the presence of specific cations such as potassium (Morris and Robinson, 1980).

Food systems can contain complex mixtures of ingredients, some of which are added to control viscosity, form a gel or act as water binding agents such as proteins and polysaccharides (Abhyankar and Auty, 2010). Texture and rheological properties

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of starch-based products are normally improved by hydrocolloids (Shi and BeMiller, 2002; Salehifar and Shahedi, 2007). Generally, rheological properties of gels depend on the molecular structure of the gelling agent. However, this can be achieved through the use of small quantities of hydrocolloids, products modification to earn higher viscosity. Usually, κ-carrageenan is deficient in hardness, springiness, water holding capacity and it is also fragile (Nishinari and Watase, 1983; Powell et al., 1982) markedly affects gel properties.

Natural polymers are known to have better biocompatibility, biodegradability, non-toxicity and easily modified ability than various synthetic materials; thus, more researches have focused on natural polymer-based hydrogels (Figuly et al., 1997). Kohyama et al. (1996) reported that KGM being a neutral polysaccharide with few acetyl groups, ionic effects on various KGM molecules was minimal, though, addition of hydrolyzed KGM to kC have been an improvement to weakness of kC alone gel properties. Previous researches in this domain have shown the effect of higher molecular weight distribution of KGM on kC gel (Nishinari and Watase, 1983; Abhyankar and Auty, 2010). Therefore, the objective of this study was to evaluate the textural and rheological properties of β-mannanase hydrolyzed KGM and kC mixed system gels as influence by system ratio, total content, molecular weight, pH and temperature.

Materials and Methods
Materials

Powdered KGM from Konjac tuber and three fractions of KGM with different molecular weights prepared by an enzymatic degradation, were kindly supplied by Jiecheng Food Additive co. Ltd (Shantou, China).The native KGM(ND) was treated with an enzyme (β-mannanase, Xiasheng Co. Ltd. Ningxia, China) for different reaction times at optimum temperature. And LM-1, LM-2, LM-3 fractions with low molecular weights were obtained. All other chemicals were obtained from the chemical reagent Co. (China) and were of food grade quality.

Methods

The kC used in this study contained substantial amount of Potassium 79.2mg/g and Sodium 43.6mg/g. the contents of minerals in both ND and hydrolysed KGM were far behind to that of kC (Table 1 (supplementary information)), this clearly indicates that their minerals content have no or little influence on the kC gel properties, even though minerals mainly affect the elastic modulus of gels. Konjac glucomannan (ND and LM-2) and kC gels were prepared at pH 3.0-9.0 using Na_2HPO_4-Ctric acid buffer with 40% KGM and total content (1.5% (w/w).

Table 1. Minerals analysis for native konjac glucomannan (ND) Hydrolyzed KGM (LM-2) and κappa-carrageenan (kC).

Sample	LM-2	ND	KC
Fe (μg/g)	18.7	25.4	<1.0
Na (mg/g)	1.64	1.94	43.6
K (mg/g)	1.97	2.33	79.2
Mg (mg/g)	4.29	5.43	7.45
Ca (mg/g)	3.69	3.82	6.57

Molecular weight determination

Molecular weight of each KGM fraction was determined by High Performance Gel Filtration Chromatography (HPGFC) under the chromatographic condition of Column: Ultrahydrogel™ Linear 300mm×7.8m mid×2 Mobile phase: 1mol/L $NaNO_3$ Flow: 0.9 ml/min Column temperature: 45℃. Sample preparation: Samples were dissolved in mobile phase, filtered with a microfiltration membrane for injection and the standards used in molecule weight calibration curve were: MW2000000, MW133800, MW21400, and MW250.

Texture and springiness measurement

The KGM solution was prepared by stirring mixed KGM powder with water continuously at the room temperature. Considering the ratio and the total content, κ-carrageenan was added into the solution and the mixture was heated in a water bath at 75±1°C for a certain time (according to experimental design) with continuous stirring. Then the gel stands at room temperature for twelve hours.

The texture was determined using the Texture Profile Analysis method (three replicates per treatment) using TA.XTplus Texture Analyzer (Lotun Science Co. Ltd, Beijing, China). Samples were compressed with a P/50 probe at pre-test speed: 2.0 mm/s test speed: 1.0 mm/s post-test speed: 5.0 mm/s. The deformation level was 40% of the original sample height and the gels were compressed twice.

Hardness was expressed as the maximum force exerted during the first compression cycle. Springiness was determined by adaptation of the AACC (1986) method into a 'Hold Until Time' test by using the Texture Analyzer.

Rheological measurement

The sample was prepared like in the texture measurement. KGM and kC were mixed and the

total content was adjusted to 1.0% (w/w). Rheological measurements were performed using an AR-G2 Rotary Rheometer from TA Company of USA equipped with cone plate geometry of 40 mm diameter (cone angle 4°, gap 108 micro m), at various temperatures and frequencies in the linear viscoelastic regime The surface of the sample was covered with silicone oil in order to prevent drying.

Strain sweep tests were performed from 0.1% to 100% at standard frequency of 10 rad/s at 25±1℃ (Figure 1) and used for subsequent analysis in this linear regime. The frequency sweeps tests were performed from 0.1 to 100 rad/s of angular frequency, at a strain of 1% in the 25±1℃. Lastly, the temperature sweep tests was carried out by heated the gel from 20 to 75±1°C with 1.0°C/min increment using 1% strain and the 10 rad/s frequency. Experimental data of G' and G" were recorded.
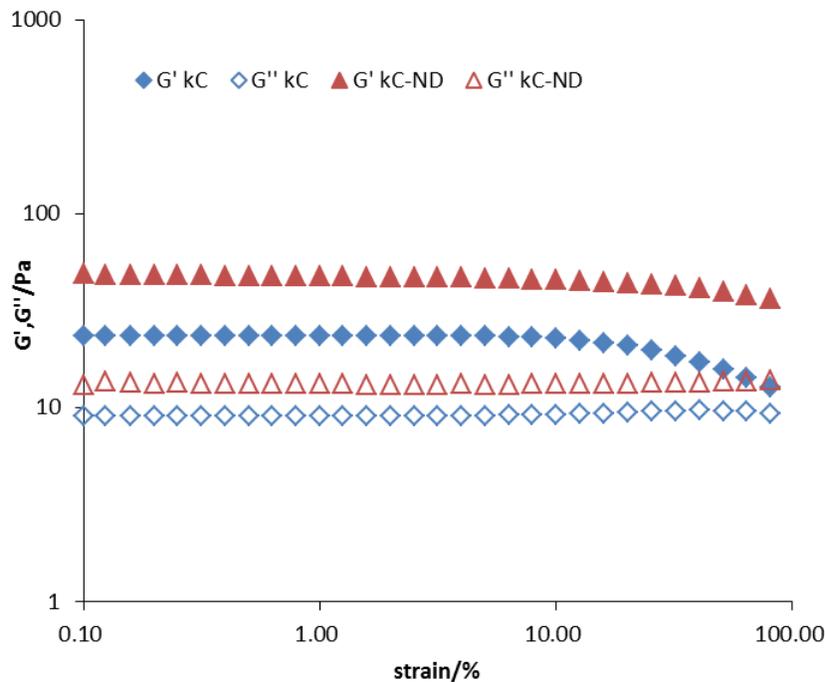
Figure 1. Strain dependence of storage modulus G' and loss modulus G"
for 10 rad/s frequency at 25±1°C.

Statistical analysis

The experimental results were evaluated by ANOVA and Tukey's multiple tests using SPSS statistical software, Version 18.0, USA. The statistical analyses were carried out within the 95% confidence interval.

Results and Discussion

Textural studies revealed that hydrolyzed KGM with different molecular weights exhibited different gelation characteristics. Our study showed that hydrolyzed KGM (LM-2) has significant textural properties against its counterpart molecular weight distribution LM-1, LM-3. Figure 1 shows that the moduli G' of kC alone and kC-ND remain larger than their G", therefore, the mixture system constituents can produce good gel with 40% KGM and 1.0% (w/w) total content.

Effect of Konjac glucomannan concentration and molecular weight on the mixed system gel

Table 2 compares the hardness and springiness of different parameters obtained by variation of KGM concentration using ND and LM-2 both selected for comparing the best molecular weight of hydrolyzed KGM (LM-2) to its native form (ND). At 40% the hardness of kC-ND and kC-LM-2 was the highest, moreover, the springiness was the lowest (Table 2). Although, the variation of KGM in gel making decreased rather than increased the texture and viscoelasticity after 40% due to the kC-KGM ratio molecular interaction, it made a major contribution to the textural properties of the gel. The springiness was inversely proportional to hardness. The kC-LM-2 exhibited higher textural and appearance than its counterpart kC-ND.

Kohyama et al. (1996) reported that the junction zone in the mixed gels was formed only by kC molecules. In other words, KGM creates weak junction zones which contribute to different rheological characteristics. Therefore, the higher the molecular weight, the longer the KGM chain. The increase in molecular weight of KGM increases the number of weak junction zones and makes the contour length of flexible chains which connect junction zones longer.

Table 2. Effect of Konjac glucomannan (KGM) concentration on gel properties.

Concentration Hardness (g)	0%	10%	20%	30%	40%	50%	60%	70%	80%
ND	410.6±32.4[g]	1934.0±107.8[c]	2134.6±182.5[bc]	2336.5±115.7[b]	2762.8±104.4[a]	1539.0±57.7[d]	1057.6±84.2[e]	769.6±84.9[f]	393.1±40.7[g]
LM-2	410.6±32.4[g]	2163.1±139.0[d]	2325.8±97.3[dc]	2634.6±131.6[c]	3156.6±171.2[b]	2090.9±142.0[d]	1429.5±45.5[e]	1052.7±12.4[f]	428.5±27.2[g]
Springiness									
ND	0.92±0.003[d]	0.890±0.004[e]	0.887±0.002[e]	0.871±0.002[fg]	0.865±0.003[g]	0.874±0.002[f]	0.919±0.003[d]	0.949±0.003[c]	0.960±0.002[b]
LM-2	0.92±0.003[c]	0.882±0.003[e]	0.884±0.001[e]	0.864±0.004[f]	0.857±0.002[g]	0.867±0.002[f]	0.906±0.002[d]	0.950±0.002[c]	0.957±0.001[b]

ND: non-degraded Konjac glucomannan, LM-2: hydrolyzed Konjac glucomannan, total content: 1.5% (w/w), heating temperature: 75±1oC for 20 minutes.

Data is expressed as mean ± standard deviation of three determinations. Mean with different letters within the row are significantly different ($P < 0.05$).

Thus, the hardness of the gel with bigger molecular weight KGM will be higher than the smaller molecular weight KGM. Figure 2 (supplementary information) shows that LM-2 has the highest hardness and closely the slowest springiness which lays our choice when compares to others fractions of hydrolyzed KGM. Indeed, the KGM chain were been shortened through this β-mannanase hydrolysis. This is probably due to the enzyme and pH factors that change the structure of the mixed gels (Chen et al., 2006; Li et al., 2004).

The experimental data of frequency dependence (G') and (G'') moduli for with 1% strain at 25±1°C of kC alone, kC-ND and kC-LM-2 KGM at frequency consistency obtained by Mixolab is shown in Figure 3. The values of G' remained higher than G'' throughout the frequency range indicating a better gel structure. G' and G'' values increased with increasing angular frequency from 1 to 100 rad/s. The slight increase noticed for kC can be attributed to the absence of binding agents; however, kC-ND and kC-LM-2 KGM are more stable. Hence, the modulus G' was larger than G'' throughout the experimentally-accessible frequency range and both moduli were little dependent on frequency showing a linear regime (Kohyama et al., 1996; Penroj et al., 2005).
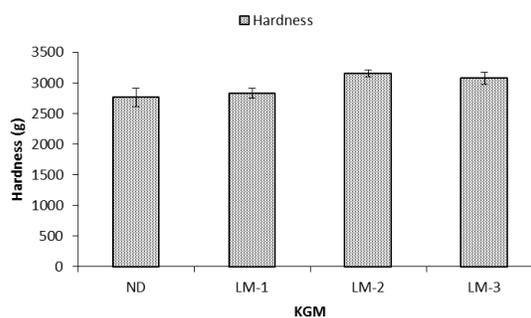
Figure 2. Effect of Konjac glucomannan (KGM) molecular weight on the gel hardness properties.

Table 3. Effect of total content on the final gel properties.

Total Content Hardness (g)	1.0%	1.2%	1.4%	1.6%	1.8%	2.0%
ND	739.7±77.1[f]	1568.4±157.2[e]	2341.7±89.3[d]	2957.4±98.0[c]	3510.6±59.1[b]	4020.5±146.4[a]
LM-2	1120.8±77.5[f]	1855.8±101.2[e]	2513.8±71.5[d]	3311.6±55.4[c]	3827.5±111.5[b]	4397.8±67.3[a]
Springiness						
ND	0.913±0.002[a]	0.897±0.003[b]	0.889±0.002[c]	0.876±0.003[d]	0.864±0.002[e]	0.849±0.003[f]
LM-2	0.902±0.002[a]	0.898±0.001[b]	0.884±0.002[c]	0.869±0.002[d]	0.858±0.003[e]	0.843±0.004[f]

ND: non-degraded Konjac glucomannan , LM-2: hydrolysed Konjac glucomannan. KGM concentration: 40%, heating temperature: 75±1oC for 20 minutes.

Data is expressed as mean ± standard deviation of three determinations. Mean with different letters within the row are significantly different (P < 0.05).
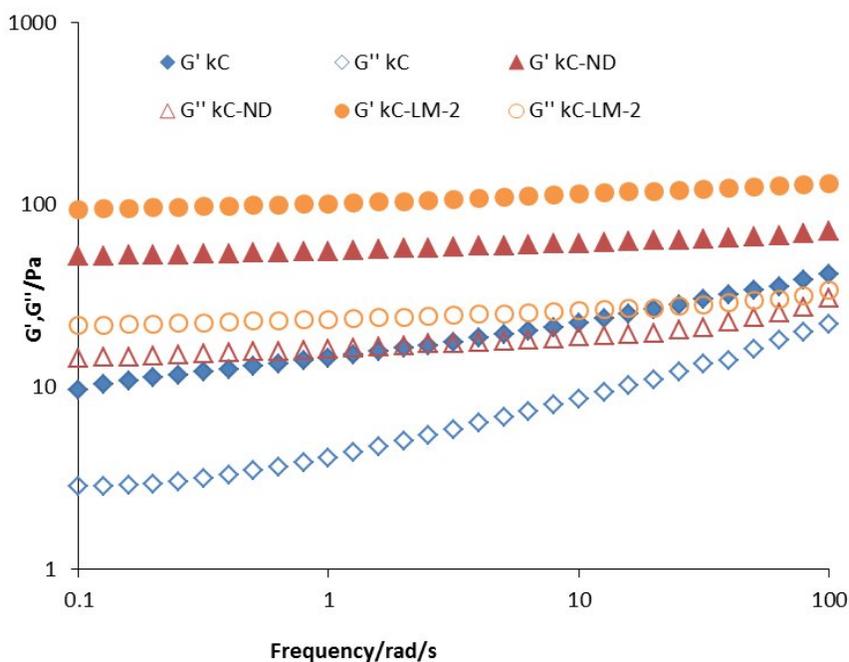
Figure 3. Frequency dependence of storage modulus G' and lost modulus G" at 1% strain at 25±1°C.

Effect of total content on the gel properties

Textural determination of KGM mixed with kC revealed significant (P<0.05) increase in hardness with increase of total content and conversely, showed significant (P<0.05) decrease in springiness (Table 3).

Table 3 shows that both hydrolyzed KGM and native KGM observed the same behavior with addition of total content in the mixed gel preparation. Native KGM is known as a gum with very high viscosity (Nishinari et al., 1992; Jacon et al., 1993). The enzyme treatment increased the ratio of lower molecular weight polymers, however, the hydrolyzed fraction LM-2 showed higher hardness than the native KGM and slightly lower springiness than the ND (Table 3). Previous studies such as x-ray diffraction have demonstrated that there is weak interaction between κappa carrageenan and KGM mixture molecules (Cheetham and Mashimba, 1988). Furthermore, it was concluded that KGM was not incorporated into κappa carrageenan crystalline junction zones. This phenomenon could be cause by the gelation rate of native KGM due to the high viscosity, which seems to inhibit the aggregation of the molecules and further delay the formation of junction zones.

Effect of temperature difference on the gel moduli

The weak gel behavior is the intermediate rheological behavior between that of the concentrated polymer solution and that of the elastic or true gel. True gels are formed by a sufficient number of junction zones in kC solutions in the presence of sufficient salts (Nishinari et al., 1996; Penroj et al., 2005). Throughout, the temperature heating, the behavior was typical of a concentrated polymer solution and those curves (G' and G") were less dependent on temperature after 50±0.2°C as it is shown in Figure 4. The kC-LM-2 gel exhibited significant G' at early temperature rising but at higher temperature (Figure 4) the G' drop drastically leaving the G" larger than G', having a melting point of 42±2°C. Therefore, this implies the temperature dependence on the gel properties. Moreover, similar behaviors were observed in the mixture systems kC-ND and kC gel alone where the kC modulus G' dropped sharply at earlier temperature of 28±0.2°C and G'= G" at 34±0.2°C which implies the kC melting point. This data corroborate with previous findings reported by Nishinari et al. (1996).

Effect of pH on the gel hardness

The samples hardness increases up to pH 5 and then began to decrease slightly and stabilized until pH 9.0 (Figure 5). The variation of Konjac glucomannan (ND and LM-2) and kC gels properties at pH 3.0-9.0 indicate the influence of pH on the gel preparation. Meng et al. (2003) reported that κappa-carrageenan is stable in the alkalinity and neutral environment, and start to hydrolyze below pH 4, thus, the hardness of the gel become lower. The pH shift is a significant factor in the stability of mixed gels. Our results corroborate with the work of Li et al. (2004) in their study on the kinetics for enzymatic degradation of KGM. Pan et al. (2011) concluded that the alkalinity of modifiers is a dominant factor for deacetylation of KGM, which leaded to improvement of gel characteristics, such as swelling property, viscous stability and thermal stability.
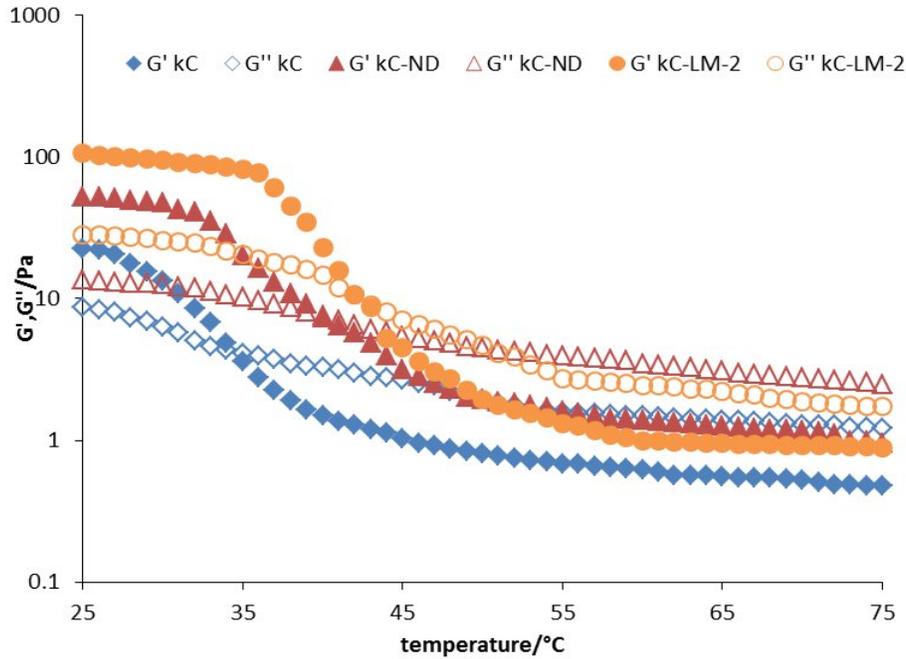
Figure 4. Temperature dependence of storage modulus G' and loss G'' during the heating process for 10 rad/s frequency and 1% strain.
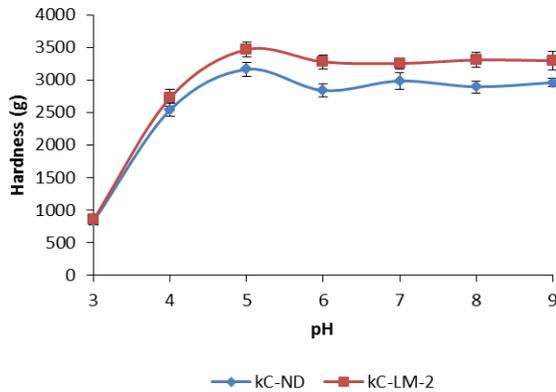
Figure 5. Effect of pH on the gel mixture system (kC-ND and kC-LM-2) hardness properties.

Effect of time at different pH on the gel hardness

The treatments were carried out with 1.5% (w/w) total content, 40% KGM concentration at 75±1°C, as shown in the Table 4, the hardness was the lowest at the pH 4 and highest at the pH 5 with the same time. Both kC-ND and kC-LM-2 exhibited the highest hardness at the time 20 minutes, when time increases the hardness reduces especially at pH 4, because the kC will be more hydrolyzed. This reflects the ability of κappa carrageenan and hydrolyzed KGM gel formation in respect with the time attributed to a segregate interaction between the two biopolymers. The possibility that the difference in hardness of the gel could be explained by the fact that difference in time scales of measurement (Penroj, 2005).

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Table 4. Effect of time at different pH on the gel hardness (g) properties.

Time (min)	10	20	30	40	50
kC-ND					
pH=4.0	1995.8±187.4[d]	1837.4±103.2[dc]	1662.5±73.2[c]	1449.9±51.8[b]	1134.2±53.1[a]
pH=5.0	2269.3±132.0[a]	2962.6±65.7[bc]	3189.4±87.7[cb]	3059.8±107.2[cb]	2944.9±67.5[bc]
pH=6.0	2374.7±96.6[a]	2937.8±84.0[b]	3185.7±78.7[c]	3105.4±68.8[bc]	3073.8±73.2[b]
LM-2					
pH=4.0	2186.5±163.0[ba]	2726.9±91.9[dc]	2511.9±52.6[d]	2277.3±108.2[d]	2027.2±90.8[c]
pH=5.0	2539.7±142.3[a]	3465.9±41.8[a]	3321.4±57.2[a]	3295.7±121.2[a]	3184.5±176.8[a]
pH=6.0	2452.4±115.9[a]	3277.3±55.2[ba]	3225.6±80.9[ba]	3194.0±71.0[ba]	3152.9±93.6[a]

ND: non-degraded Konjac glucomannan , LM-2: hydrolyzed Konjac glucomannan total content: 1.5% (w/w), KGM concentration: 40%, heating temperature: 75±1oC.

Data is expressed as mean ± standard deviation of three determinations. Mean with different letters within the row are significantly different (P < 0.05).

Conclusion

The effect the ratio of konjac glucomannan (KGM) and κappa carrageenan (kC), total gel content, molecular weight, pH and temperature on the gelation are responsible for system kC and KGM texture and rheological properties. The increase in hardness of LM-2 molecular weight KGM compare to Non-Degraded KGM was likely due to enzyme used in the hydrolyisis of native KGM, and the springiness were found to be inversely proportional to the hardness. The kC-LM-2 was found to be the prominent gel for our texture properties. Further studies are needed on specific effect of β-mannanase on this mixed system gel and its application is now being undertaken in our laboratory.

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