Journal of Multidisciplinary Engineering Science and Technology (JMEST)
ISSN: 3159-0040
Vol. 2 Issue 11, November - 2015

Optimization Of Mechanical Properties Of Carboxymethyl Cellulose, Carrageenan And/Or Xanthan Gum Gels As Alternatives Of Gelatin Softgels Capsules

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***Abstract*—Gelatin is used as a thickening, gelling, stabilizing and emulsifying agent. Due to the demand for non-animal products, recent studies involve the search of gelatin alternatives materials for softgel capsules. The aim of this study was to optimize the mechanical properties of carboxymethyl cellulose (CMC), carrageenan and/or xanthan gum gels, having gelatin gels as target, using a response surface methodology. The analyzed gelatin concentrations were 30, 40, and 50%; whereas polysaccharide concentrations were 5, 7.5, and 10%. A texture profile analysis (hardness, adhesiveness, springiness and cohesiveness) was used for measuring the mechanical properties of gels at different temperatures while cooling down (70, 60, 50, 40, and 30ºC). The response surface methodology was set up with carrageenan and CMC blend at 7.5 or 10%, at different carrageenan:CMC proportions (1:0, 0:1, 1:1, 2:1 and 3:1). For each of the studied gels systems at 30ºC, an optimization was carried out for two mechanical properties: hardness and adhesiveness. In general, hardness and adhesiveness increased as the polymer concentration increased and temperature decreased; however no trends were observed for the other two texture parameters. Regardless polymer concentration, gelatin gels texture showed an important shift in hardness and adhesiveness when temperature cooled down from 40 to 30ºC. The validation of the optimized conditions showed that there are formulations of Carrageenan:CMC that can replace gelatin systems under different proportions with good reproducibility.**

Keywords—polysaccharide gels, texture profile, gelatin soft gels

I. INTRODUCTION

Gelatin is a collagen-derived protein that is used as a functional ingredient in pharmaceutical, cosmetic and food industries. Gelatin applications include: modification of textural properties of desserts [1], encapsulation of vitamins, antioxidants and polyunsaturated fatty acids [2], microencapsulation of probiotics [3] and development of edible films and coatings [4]. Gelatin used concentrations vary from 2% in the food industry and up to 50% for soft-gel capsules production [5,6].

In recent years, there has been an increasing demand for non-animal products driven by religious beliefs, cultural traditions, health, and safety issues. Globally, scientists are in continuous search for a non-toxic, renewable and affordable ingredient or combination of ingredients that could be used as a gelatin alternative in different products. Therefore, studies concerning the use of several biopolymers from vegetable sources as gelatin alternative have been conducted recently [7, 8, 9]. Among the evaluated materials selected polysaccharides such as carboxymethyl cellulose (CMC); carrageenan, and some microbial produced gums such as xanthan gum [10] have been studied.

For several food processing technologies, mechanical properties during gel formation are of particular interest [11]. Many studies show that these properties are not only largely dependent on the polymer type, but are also greatly influenced by process temperatures [12, 13]. Mechanical properties of gelatin gels have been extensively studied; however, there are not enough studies regarding these properties during gel formation, neither for research comparing gelatin gels mechanical properties using alternative materials as gel forming agents.

In general, scientists have been analyzing gelatin concentrations from 6 to 10% [14, 15, 16]. Investigations of higher gelatin concentrations have not been documented; however, for assessing the use of gelatin alternatives in diverse applications, studies involving higher gelatin concentrations are needed. Gelatin gels show an important shift in textural properties while cooling down; hardness increases as adhesiveness decreases [17]. Among the functional and textural properties of gels used in food industries, hardness, adhesiveness, cohesiveness and springiness are some of the most important [7, 18].

Thus the objective of this study was to optimize the mechanical properties of CMC, carrageenan and/or xanthan gum gels compared to gelatin gels, using a response surface methodology (RSM).

II. MATERIALS AND METHODS

A. *Materials*

Pharmaceutical gelatin was donated (Biocaps, Mexico). Certified kosher kappa-carrageenan (Carr), carboxymethyl cellulose (CMC) and xanthan gum were purchased (CNI, France). Glycerol at 95% (RBM, Mexico) was used as plasticizer.

B. *Gel formation*

Gels were prepared in 150 ml beakers placed in a water bath at 80 ±1ºC mixing distilled water with glycerol at 10% (w/w dry polymer, to obtain 100 g of gel) and then adding the polymer, occasionally stirring for 15 minutes or until complete dissolution. The evaluated gelatin concentrations were 30, 40 or 50%; while the studied concentrations for CMC, Carr and xanthan gums were 5, 7.5 or 10%; and, 7.5 or 10% for Carr and CMC mixtures at five Carr:CMC proportions (0:1, 1:0, 1:1, 2:1, 3:1).

C. *Mechanical properties of gels*

Gels were prepared Gels' mechanical properties were measured while cooling down at room temperature from 70 to 30ºC, maintaining the gel systems submerged in a water bath for a better temperature distribution of the system. The properties of gels were determined with a texture profile analysis (TPA) using a TA-TX2 texturometer (Stable Micro Systems LTD., U.K.). A steel cylinder 10 mm flat bottom probe was used, setting the test to 12 mm penetration distance, with a pre-test, test and post-test speed of 1 mm s^{-1} [15].

The mechanical properties registered were: *hardness,* defined as the height of the force peak (N) on the first compression cycle; *adhesiveness,* the negative area after the first compression (N.s) and before the second cycle; *springiness,* defined as the ratio of the distance penetrated by the probe at the maximum force of the second compression and the distance at the maximum force of the first compression, it describes whether the material springs back to its original state once compressed; and *cohesiveness,* defined as the ratio of the area under the first compression and the second compression and it is related to the sample internal structure.

D. *Statistical analysis and optimization*

Hardness, adhesiveness, springiness, and cohesiveness were compared statistically by performing analysis of variance (ANOVA). In order to study significant main effects and interactions ($p<0.05$), a design of experiment was set up for hardness and adhesiveness individually.

Given that gelatin gels hardness and adhesiveness showed an important shift when cooling down from 40 to 30ºC, two biopolymers were selected to obtain a similar behavior for each of the studied gelatin concentrations.

A response surface methodology was set up for Carr and CMC mixtures, for each of the three studied gelatin concentrations at 30ºC, an optimization was made for hardness and adhesiveness. Statistical analyses were made using Minitab Version 16.0 statistical software (Minitab Inc., U.S.A.).

III. RESULTS AND DISCUSSION

Previous reported studies for mechanical properties of gels under the conditions used for the present study were not found; even though, some comparisons of gels adhesiveness and hardness have been made with different materials and at different conditions [7, 11, 13, 15].

Gels' hardness, adhesiveness, springiness and cohesiveness were measured and compared at different temperatures; values are shown in Tables I-IV.

A. *Hardness*

As it can be seen in Table I, at 30ºC hardness of gelatin gels at studied concentrations differ significantly ($p<0.05$). It can also be noted that at the same temperature none of the alternative polymers (CMC, carrageenan, xanthan gum) gels studied could be compared in their hardness to gels formed with gelatin at any concentration. The evaluated carrageenan concentrations formed harder gels at 30°C, while CMC and xanthan gum gels exhibited lower hardness values. At 30ºC, an increase in gelatin's concentration from 40% to 50% formed a gel 4.18 times harder; this effect cannot be observed when increasing gelatin concentration from 30% to 40%, showing that gel hardness does not follow a linear behavior as function of gelatin concentration. According to reference [11], the increase in hardness as function of gelatin concentration can be explained by a higher protein content, which improves the structural bonding of the material and thus forming stiffer gels. Based on the statistical analysis, forming gel material ($p=0.001$), concentration ($p=0.002$), temperature ($p=0.000$), and material and concentration interaction ($p=0.002$) are statistically significant to hardness of formed gels.

It is important to observe that hardness of gelatin gels increased markedly when temperature dropped from 40 to 30ºC. Hardness of 30% gelatin gels was more than 65 times greater at 30ºC (164.20×10^{-2} N) than at 40ºC (2.52×10^{-2} N); 40% gelatin gels were 105 times harder at 30ºC, and for gelatin at 50% the change in temperature caused an increase in hardness of 243 times.

During gelatin gel formation, glycine, proline and hydroxyproline form regions where water is trapped. At higher temperatures, gelatin molecules are presented as random coils, which begin to form triple helical junction zones as the gel cools down [19]. This gelation mechanism, could explain the change in gelatin gel mechanical properties when cooling down from 40 to 30ºC observed in the present study. Carrageenan gels also undergo through a coil-helix transition when cooling down; however, unlike gelatin, it is a double helix structure [20, 17]. As it can be observed in Table I, unlike gelatin gels the raise in hardness values of carrageenan gels was gradual throughout the studied range of temperatures.

TABLE I. GEL HARDNESS (N X 10^{2}) MEASURED WHEN COOLING DOWN DIFFERENT POLYMERS AT DIFFERENT CONCENTRATIONS

System	30°C	40°C	50°C	60°C	70°C
Gelatin					
30%	164.20 ± 48.19 a $_{\mathrm{I}}$	2.52 ± 0.23 a $_{\mathrm{II}}$	2.33 ± 0.21 a $_{\mathrm{II}}$	2.53 ± 0.12 a $_{\mathrm{II}}$	2.01 ± 0.17 a $_{\mathrm{III}}$
40%	275.98 ± 22.26 b $_{\mathrm{I}}$	2.63 ± 0.34 a $_{\mathrm{II}}$	2.55 ± 0.18 a $_{\mathrm{II}}$	2.35 ± 0.20 a $_{\mathrm{II}}$	2.34 ± 0.24 a $_{\mathrm{II}}$
50%	1155.67 ± 158.10 c $_{\mathrm{I}}$	4.75 ± 0.40 b $_{\mathrm{II}}$	3.68 ± 0.46 b $_{\mathrm{III}}$	3.69 ± 0.61 b $_{\mathrm{III}}$	2.87 ± 0.28 b $_{\mathrm{IV}}$
Carrageenan					
5%	1556.12 ± 26.19 d $_{\mathrm{I}}$	200.70 ± 51.33 c $_{\mathrm{II}}$	14.19 ± 3.91 c $_{\mathrm{III}}$	5.41 ± 0.65 c $_{\mathrm{IV}}$	4.56 ± 0.71 c $_{\mathrm{IV}}$
7.5%	2147.81 ± 105.68 e $_{\mathrm{I}}$	1439.44 ± 111.17 d $_{\mathrm{II}}$	121.58 ± 30.98 d $_{\mathrm{III}}$	24.98 ± 2.64 d $_{\mathrm{IV}}$	21.39 ± 3.67 d $_{\mathrm{IV}}$
10%	3444.83 ± 207.13 f $_{\mathrm{I}}$	1920.74 ± 151.97 e $_{\mathrm{II}}$	659.59 ± 200.94 e $_{\mathrm{III}}$	168.64 ± 36.03 e $_{\mathrm{IV}}$	73.17 ± 19.56 e $_{\mathrm{V}}$
CMC					
5%	2.72 ± 0.30 g $_{\mathrm{I}}$	2.58 ± 0.19 a $_{\mathrm{I}}$	2.37 ± 0.20 a $_{\mathrm{I}}$	2.58 ± 0.27 a $_{\mathrm{I}}$	2.42 ± 0.30 a $_{\mathrm{I}}$
7.5%	5.93 ± 0.49 h $_{\mathrm{I}}$	4.45 ± 0.64 b $_{\mathrm{II}}$	3.41 ± 0.31 b $_{\mathrm{III}}$	3.53 ± 0.35 b $_{\mathrm{III}}$	2.47 ± 0.35 a,b $_{\mathrm{IV}}$
10%	17.87 ± 1.23 i $_{\mathrm{I}}$	13.14 ± 1.68 f $_{\mathrm{II}}$	9.36 ± 1.07 c $_{\mathrm{III}}$	7.09 ± 0.61 f $_{\mathrm{IV}}$	5.42 ± 0.54 c $_{\mathrm{V}}$
Xanthan gum					
5%	21.05 ± 3.15 i $_{\mathrm{I}}$	20.60 ± 3.79 g $_{\mathrm{I}}$	19.02 ± 2.18 f $_{\mathrm{I, II}}$	16.62 ± 1.62 g $_{\mathrm{II}}$	14.08 ± 0.62 f $_{\mathrm{III}}$
7.5%	46.24 ± 3.21 j $_{\mathrm{I}}$	40.27 ± 1.99 h $_{\mathrm{II}}$	44.23 ± 4.67 g $_{\mathrm{II}}$	38.83 ± 4.04 h $_{\mathrm{II}}$	39.38 ± 6.82 g $_{\mathrm{I, II}}$
10%	64.32 ± 9.62 k $_{\mathrm{I}}$	53.78 ± 7.60 i $_{\mathrm{I}}$	52.27 ± 8.97 g $_{\mathrm{I}}$	54.08 ± 13.05 i $_{\mathrm{I, II}}$	41.21 ± 0.61 e,g $_{\mathrm{II}}$

Each data point represents the mean of three experiments with two replicates for each system ±SD (n=6). Values within a column followed by different letter are significantly different (p≤0.05). Values within a row followed by the different subscripts are significantly different (p≤0.05).

At temperatures above 30ºC, hardness of gelatin gels at 30 or 40% were significantly equal (p>0.05) to gels formed with CMC at 5% at the same temperature range. Besides, gels formed with 50% gelatin were significantly equal (p>0.05) to those formed with 7.5% CMC. None of the other polymers at the concentrations evaluated are significantly similar (p<0.05) to gelatin gels hardness values. Hardness of 5%CMC gels did not depend on temperature; however, the same material at higher concentrations (7.5 or 10%) presented an increase in hardness as temperature dropped.

In Table I, it can also be observed that hardness of xanthan gum gels did not undergo many changes as function of temperature. Regardless, at all given temperatures, there is a trend that shows an increase in hardness as xanthan gum concentration increased. According to reference [11], compared to other biopolymer, xanthan formed stiffer gels; in the present study this corresponds to hardness values exhibited at temperatures higher than 50ºC.

B. Adhesiveness

Unlike hardness, as it can be seen in Table II, adhesiveness at 30ºC of gelatin gels at 40 or 50% did not differ significantly (p>0.05) from 7.5% carrageenan or 10% CMC gels. However, none of the analyzed systems showed adhesiveness values similar to 30% gelatin gels.

In a previous study it was demonstrated that adhesiveness showed no significant difference as the concentration of gelatin increased from 20 to 30% [18]. This behavior can be seen at 30ºC, where there was no significant difference (p>0.05) in adhesiveness of 40% (-128.96 x 10^{-2} N.s) and 50% (-121.60 x 10^{-2} N.s) gelatin gels. However, it can be seen that increasing gelatin concentration from 30% to 40% caused an increase in adhesiveness. In the range of the studied temperatures, in most of the cases, higher concentrations of gelatin led to more adhesive gels. The concentration (p=0.034) and gel formation material (p=0.027); as well as their interaction (p=0.000) have a significant effect in the adhesiveness.

Carrageenan gels adhesiveness does not seem to have a defined trend between concentration and temperature. Carrageenan gels at 10% are adhesive only at 40 and 50ºC; however, the property could not be measured at 30ºC because of the high hardness value displayed by the formed gel.

On the other hand, at the studied conditions, adhesiveness of CMC gels increased as concentration increased and temperature decreased; this effect was less obvious for gels formed using 5% of the polysaccharide. While xanthan gum gels were the most adhesive at the evaluated temperatures and almost at every concentration, except for 5% gum at 30ºC (-166.56 x 10^{-2} N.s) that was not significantly different, (p>0.05) to the adhesiveness of 5% carrageenan gels (-212.47 x 10^{-2} N.s).

TABLE II. Gel adhesiveness (N s x 10^2) measured when cooling down different polymers at different concentrations

System	30°C	40°C	50°C	60°C	70°C
Gelatin					
30%	-107.15 ± 6.25 a $_{\mathrm{I}}$	-12.11 ± 2.36 a $_{\mathrm{II}}$	-9.72 ± 2.86 a $_{\mathrm{II}}$	-1.91 ± 0.59 a $_{\mathrm{III}}$	-3.14 ± 0.85 a $_{\mathrm{III}}$
40%	-128.96 ± 7.55 b $_{\mathrm{I}}$	-23.76 ± 3.47 b $_{\mathrm{II}}$	-13.46 ± 2.43 a $_{\mathrm{III}}$	-15.35 ± 1.09 b $_{\mathrm{III}}$	-9.90 ± 0.92 b $_{\mathrm{IV}}$
50%	-121.60 ± 8.65 b $_{\mathrm{I}}$	-99.35 ± 14.12 c $_{\mathrm{II}}$	-23.29 ± 4.45 b,c $_{\mathrm{III}}$	-20.41 ± 0.92 c $_{\mathrm{IV}}$	-17.22 ± 4.23 c $_{\mathrm{IV}}$
Carrageenan					
5%	-212.47 ± 34.80 c $_{\mathrm{I}}$	-8.20 ± 3.39 a $_{\mathrm{II}}$	-24.30 ± 5.99 b $_{\mathrm{III}}$	-22.78 ± 4.94 b,c $_{\mathrm{III}}$	-28.74 ± 4.38 d $_{\mathrm{III}}$
7.5%	-128.22 ± 1.07 b $_{\mathrm{I}}$	-50.20 ± 9.01 d $_{\mathrm{II}}$	-55.41 ± 3.82 d $_{\mathrm{III}}$	-28.62 ± 4.83 d $_{\mathrm{IV}}$	-5.20 ± 1.44 a $_{\mathrm{V}}$
10%	-0.12 ± 0.27 d $_{\mathrm{I}}$	-35.34 ± 9.91 d $_{\mathrm{II}}$	-108.03 ± 24.03 e $_{\mathrm{III}}$	0.00 ± 0.00 e $_{\mathrm{I}}$	0.00 ± 0.00 e $_{\mathrm{I}}$
CMC					
5%	-21.13 ± 5.66 e $_{\mathrm{I}}$	-20.95 ± 3.62 b $_{\mathrm{I}}$	-18.22 ± 2.61 c $_{\mathrm{I}}$	-19.53 ± 2.07 c $_{\mathrm{I}}$	-13.85 ± 2.59 f $_{\mathrm{II}}$
7.5%	-53.10 ± 3.74 f $_{\mathrm{I}}$	-44.79 ± 3.53 d $_{\mathrm{II}}$	-30.15 ± 3.07 b $_{\mathrm{III}}$	-21.47 ± 2.64 c $_{\mathrm{III}}$	-14.55 ± 3.22 f $_{\mathrm{IV}}$
10%	-128.97 ± 5.68 b $_{\mathrm{I}}$	-104.74 ± 4.37 c $_{\mathrm{II}}$	-69.52 ± 2.96 f $_{\mathrm{III}}$	-67.25 ± 4.95 f $_{\mathrm{III}}$	-50.03 ± 5.29 g $_{\mathrm{IV}}$
Xanthan gum					
5%	-166.59 ± 23.55 c $_{\mathrm{I}}$	-181.82 ± 42.57 e $_{\mathrm{I}}$	-168.11 ± 20.27 g $_{\mathrm{I}}$	-122.66 ± 11.07 g $_{\mathrm{II}}$	-88.24 ± 6.19 h $_{\mathrm{III}}$
7.5%	-304.30 ± 23.74 g $_{\mathrm{I}}$	-302.70 ± 8.04 f $_{\mathrm{I}}$	-259.16 ± 4.08 h $_{\mathrm{II}}$	-253.66 ± 23.15 h $_{\mathrm{II}}$	-251.46 ± 26.44 i $_{\mathrm{II}}$
10%	-410.96 ± 89.02 h $_{\mathrm{I}}$	-410.10 ± 45.49 g $_{\mathrm{I}}$	-413.39 ± 27.40 i $_{\mathrm{I}}$	-328.73 ± 35.37 i $_{\mathrm{I}}$	-61.58 ± 3.36 j $_{\mathrm{II}}$

Each data point represents the mean of three experiments with two replicates for each system ±SD (n=6). Values within a column followed by different letter are significantly different ($p \le 0.05$). Values within a row followed by the different subscripts are significantly different ($p \le 0.05$).

The adhesiveness of the different formulated gels did not follow the same change while cooling down as gelatin gels did. For example, the adhesiveness of 50% gelatin gel at 40°C was not significantly different (p>0.05) to that of CMC gel at 10%; however, at 50º, 60º and 70ºC there were significant differences (p<0.05) between the adhesiveness displayed by both biopolymers gels at the same temperature. From this analysis, it is demonstrated that temperature (p=0.000) has a significant effect for this mechanical property.

C. *Springiness*

The property that measures how well the gel springs back after the first compression is springiness. In Table III, it can be observed that, similarly to hardness, there is an important change in springiness when temperature gels decreased from 40 to 30ºC for 30 or 40% gelatin gels concentration. It can also be observed that in gelatin systems an increase in the concentration from 30 to 40% did not causes a significant increment (p>0.05) in springiness. However, increasing gelatin concentration from 40 to 50% did cause a significantly (p<0.05) difference in springiness values, especially at 40 and 50ºC.

For xanthan gum neither concentration nor temperature had effect on the springiness gel property. It can also be observed that at 30ºC springiness values of gelatin systems were not significantly different (p>0.05) to those exhibited by xanthan gum at the studied concentrations. However for the other evaluated temperatures there were significant differences between xanthan gum and gelatin gels springiness. In general, as observed for hardness, springiness of gelatin gels at 30 or 40% above 30ºC was similar to CMC at 5%.

Springiness cannot be described as function of temperature nor concentration, except for springiness of CMC systems, which in most cases increased as concentration increased.

D. *Cohesiveness*

A relation between concentration and cohesiveness could not be established for any material (Table IV). At 30ºC and 70ºC there was no significant difference (p>0.05) of the three studied gelatin concentrations in cohesiveness. Cohesiveness of gelatin gels at 40% is not significantly different (p>0.05) to xanthan gum gels at 10% at the studied temperatures. On the other hand at the evaluated temperatures, cohesiveness of gelatin at 50% is not significantly different (p>0.05) to CMC at 7.5% and in some cases to CMC 10%.

Unlike hardness, adhesiveness and springiness there is not a specific trend that relates the cohesiveness with temperature for none of the studied materials.

In general, there was a trend between temperature and hardness, as well as temperature and adhesiveness; since the hardness increased as the temperature decreased. It can also be noted that as the polymer concentration was raised, hardness, adhesiveness and springiness, in some cases, also increased. It was found that properties are function of temperature, material and concentration. It can also be concluded from the present study that xanthan gum, CMC and carrageenan gels properties differ from gelatin gels. In general, xanthan gels were more adhesive and showed higher springiness values; CMC gels were more cohesive; and carrageenan formed stiffer gels. Even when these materials have been

TABLE III. GEL SPRINGINESS MEASURED WHEN COOLING DOWN DIFFERENT POLYMERS AT DIFFERENT CONCENTRATIONS

System	30°C		40°C		50°C		60°C		70°C	
Gelatin										
30%	0.91 ± 0.02	a	0.44 ± 0.10	a	0.37 ± 0.04	a	0.48 ± 0.08	a	0.45 ± 0.07	a
40%	0.87 ± 0.06	a,b	0.53 ± 0.09	a	0.45 ± 0.10	a,b	0.49 ± 0.11	a	0.47 ± 0.14	a,d
50%	0.85 ± 0.10	a,b	0.83 ± 0.10	b,e	0.63 ± 0.06	c	0.66 ± 0.09	a,b	0.66 ± 0.09	a,b,c
Carrageenan										
5%	0.95 ± 0.04	a,c	0.83 ± 0.12	b,c,d	0.76 ± 0.06	d,e	0.76 ± 0.06	b,c,d	0.69 ± 0.07	c
7.5%	0.88 ± 0.13	a,b	0.54 ± 0.10	a	0.76 ± 0.14	c,d,f,g	0.86 ± 0.09	c,e	0.76 ± 0.13	c,d
10%	0.79 ± 0.06	b	0.92 ± 0.06	e	0.79 ± 0.18	c,d,f,g	0.61 ± 0.11	a,d	0.41 ± 0.04	a
CMC										
5%	0.46 ± 0.10	d	0.51 ± 0.04	a	0.47 ± 0.05	b	0.58 ± 0.10	a	0.54 ± 0.07	a
7.5%	0.81 ± 0.05	b	0.73 ± 0.07	c,e	0.56 ± 0.06	c	0.59 ± 0.09	a	0.56 ± 0.10	a,c
10%	0.98 ± 0.02	b,c	0.96 ± 0.00	d,e	0.93 ± 0.02	f	0.90 ± 0.04	e	0.85 ± 0.06	b,d
Xanthan gum										
5%	0.90 ± 0.02	a	0.90 ± 0.02	b,e	0.90 ± 0.03	f,h	0.90 ± 0.04	e	0.86 ± 0.05	b,d
7.5%	0.92 ± 0.03	a	0.94 ± 0.02	b,e	0.90 ± 0.02	f,h	0.91 ± 0.02	e	0.89 ± 0.02	b,d
10%	0.90 ± 0.02	a	0.88 ± 0.03	b,e	0.88 ± 0.00	e,g,h	0.90 ± 0.02	e	0.84 ± 0.09	b,d

Each data point represents the mean of three experiments with two replicates for each system ±SD (n=6). Values within a column followed by different letter are significantly different ($p \leq 0.05$).

TABLE IV. GEL COHESIVENESS MEASURED WHEN COOLING DOWN DIFFERENT POLYMERS AT DIFFERENT CONCENTRATION

System	30°C		40°C		50°C		60°C		70°C	
Gelatin										
30%	0.84 ± 0.10	a,b	0.70 ± 0.04	a	0.66 ± 0.06	a,b	0.68 ± 0.03	a	0.83 ± 0.11	a,b,c
40%	0.95 ± 0.05	a,c,f	0.87 ± 0.05	b	0.81 ± 0.13	c,d	0.79 ± 0.11	b,c	0.72 ± 0.13	a,b
50%	0.96 ± 0.02	a,c,f	1.00 ± 0.27	b,c,d	0.96 ± 0.07	e,f	0.92 ± 0.09	b,d	0.72 ± 0.02	a,b
Carrageenan										
5%	0.35 ± 0.04	d	0.40 ± 0.10	e	0.73 ± 0.02	d,g	0.95 ± 0.04	d,e,f	1.00 ± 0.12	c,d
7.5%	0.72 ± 0.10	b,c,d	0.40 ± 0.08	e	0.52 ± 0.04	a	0.69 ± 0.11	a,c,g,h	0.65 ± 0.10	a,e,f
10%	0.61 ± 0.21	a,c,e	0.63 ± 0.18	a,c,e	0.33 ± 0.08	h	0.36 ± 0.05	i	0.41 ± 0.14	f
CMC										
5%	0.71 ± 0.08	d,e	0.75 ± 0.04	a,f	0.77 ± 0.07	c,g	0.86 ± 0.09	b,e	0.84 ± 0.08	b,g
7.5%	0.97 ± 0.04	c	0.88 ± 0.11	b	0.81 ± 0.09	c,e,g	0.80 ± 0.05	b,g,j	0.81 ± 0.10	a,b,c
10%	1.00 ± 0.02	f	1.00 ± 0.05	d	1.00 ± 0.06	f	1.00 ± 0.03	f	1.00 ± 0.10	d
Xanthan gum										
5%	0.82 ± 0.08	a,d	0.77 ± 0.13	a,b,f	0.78 ± 0.07	c	0.84 ± 0.05	b,g	0.87 ± 0.06	c,g
7.5%	0.69 ± 0.03	d	0.80 ± 0.07	b,f	0.72 ± 0.07	b,c,g	0.74 ± 0.05	a,c,j	0.67 ± 0.03	e
10%	0.79 ± 0.11	a,d	0.85 ± 0.05	b	0.72 ± 0.07	b,c,g	0.74 ± 0.02	c,h,j	0.75 ± 0.05	a,b

Each data point represents the mean of three experiments with two replicates for each system ±SD (n=6). Values within a column followed by different letter are significantly different ($p \leq 0.05$).

widely studied as gel forming agents, there is not reported information at the concentrations proposed in the present study.

E. Carrageenan and CMC gels

Due to the statistical analysis of the studied mechanical properties, carrageenan and CMC were selected to evaluate the behavior of gels when using mixtures of these on behalf to comply with gelatin behavior; shift in hardness when cooling down from 40 to 30ºC and similar adhesiveness values at the given temperatures. Carrageenan (Carr) was selected because none of the other studied polymers formed as hard gels as gelatin did at 30ºC; while CMC gels hardness at temperatures higher than 30ºC was not significantly different to gelatin gels. To compensate for the lack of adhesiveness that carrageenan gels had and the higher hardness values at low temperatures, CMC was added.

Therefore, the studied concentrations for Carr:CMC were 7.5 and 10%, while the proposed Carr:CMC ratios were 0:1, 1:0, 1:1, 2:1 and 3:1 (0, 100, 50, 66 and 75 expressed as carrageenan percent respectively).

Hardness and adhesiveness of carrageenan and CMC gels at different conditions can be observed in Table V and Table VI. Since cohesiveness, and in most cases springiness, did not show a trend in the previous section and neither in this part of the study, these results are not shown.

As it can be observed in Table V, formulating with 7.5% pure carrageenan led to gels even 11 times harder than hardness exhibited by gels when CMC was added at a small proportion of 3:1. On the other hand, at 10% pure carrageenan, hardness was around 5 times higher compared to Carr:CMC 3:1.

It is also important to note the effect that adding CMC had in adhesiveness. As it can be seen in Table VI, at the studied conditions, gels formulated with the blend Carr:CMC were more adhesive compared to single polysaccharide formulations.

TABLE V. GEL HARDNESS (N X 10^2) MEASURED WHEN COOLING DOWN CARRAGEENAN AND CARBOXYMETHYL CELLULOSE SYSTEMS MIXTURES AT DIFFERENT CONCENTRATIONS

System	30°C	40°C	50°C	60°C	70°C
7.5%					
Carr:CMC (0:1)	5.93 ± 0.49	4.45 ± 0.64	3.41 ± 0.31	3.53 ± 0.35	2.47 ± 0.35
Carr:CMC (1:1)	124.13 ± 11.39	32.43 ± 2.96	6.16 ± 0.58	4.13 ± 0.21	3.97 ± 0.32
Carr:CMC (2:1)	255.75 ± 17.48	105.73 ± 7.40	13.80 ± 1.79	5.27 ± 0.49	5.06 ± 0.43
Carr:CMC (3:1)	354.32 ± 7.34	127.92 ± 7.24	17.07 ± 2.48	7.73 ± 0.13	5.38 ± 0.16
Carr:CMC (1:0)	2147.81 ± 105.68	1439.44 ± 11.17	121.58 ± 30.89	24.98 ± 2.64	21.39 ± 3.67
10%					
Carr:CMC (0:1)	17.87 ± 1.23	13.14 ± 1.68	9.36 ± 1.07	7.09 ± 0.61	5.42 ± 0.54
Carr:CMC (1:1)	186.86 ± 19.24	63.39 ± 4.50	20.48 ± 1.10	11.16 ± 0.60	7.98 ± 0.92
Carr:CMC (2:1)	601.72 ± 15.24	233.38 ± 21.06	147.06 ± 16.40	24.88 ± 4.03	17.47 ± 2.14
Carr:CMC (3:1)	632.13 ± 44.52	432.11 ± 20.41	265.23 ± 18.73	51.13 ± 6.13	26.18 ± 2.80
Carr:CMC (1:0)	3444.83 ± 207.13	1920.74 ± 151.97	659.59 ± 200.94	168.64 ± 36.03	73.17 ± 19.56

Each data point represents the mean of three experiments with two replicates for each system ±SD (n=6).

TABLE VI. GEL ADHESIVENESS (N S X 10^2) MEASURED WHEN COOLING DOWN CARRAGEENAN AND CARBOXYMETHYL CELLULOSE SYSTEMS MIXTURES AT DIFFERENT CONCENTRATIONS

System	30°C	40°C	50°C	60°C	70°C
7.5%					
Carr:CMC (0:1)	-53.10 ± 3.74	-44.79 ± 3.53	-30.15 ± 3.07	-21.47 ± 2.64	-14.55 ± 3.22
Carr:CMC (1:1)	-536.50 ± 22.35	-80.31 ± 3.89	-57.51 ± 3.25	-40.51 ± 3.99	-37.63 ± 0.26
Carr:CMC (2:1)	-542.28 ± 37.14	-93.13 ± 8.13	-68.25 ± 4.88	-51.41 ± 2.21	-45.71 ± 1.23
Carr:CMC (3:1)	-581.75 ± 7.94	-161.28 ± 17.13	-102.10 ± 8.58	-74.12 ± 4.26	-43.47 ± 4.79
Carr:CMC (1:0)	-128.22 ± 1.07	-50.20 ± 9.01	-55.41 ± 3.82	-28.62 ± 4.83	-5.20 ± 1.44
10%					
Carr:CMC (0:1)	-128.97 ± 5.68	-104.74 ± 4.37	-69.52 ± 2.96	-67.25 ± 4.95	-50.03 ± 5.29
Carr:CMC (1:1)	-540.53 ± 22.09	-321.08 ± 12.92	-100.19 ± 8.18	-73.33 ± 2.71	-59.44 ± 1.57
Carr:CMC (2:1)	-1373.74 ± 111.58	-468.53 ± 65.52	-148.35 ± 11.62	-121.81 ± 11.34	-80.01 ± 2.97
Carr:CMC (3:1)	-1611.39 ± 49.25	-851.70 ± 135.56	-384.84 ± 12.00	-76.62 ± 1.12	-127.61 ± 23.74
Carr:CMC (1:0)	-0.12 ± 0.27	-35.34 ± 9.91	-108.03 ± 24.03	0.00 ± 0.00	0.00 ± 0.00

Each data point represents the mean of three experiments with two replicates for each system ±SD (n=6)

F. *Predictive models and validation*

Second-order polynomial models were proposed to predict each gel property (hardness and adhesiveness). Given that the coefficients of the models were relatively low, mathematical transformations of the responses were made, in order to improve the predictive model certainty and decrease variability. The selected transformations can be seen in equations 1 and 2. The R^2 coefficients of the adjusted models were 0.9603 and 0.8153 for hardness and adhesiveness, respectively.

Hardness prediction was less accurate for 10% polymer concentration compared to 7.5%. On the other hand, considering temperature the higher residuals values were obtained at 30ºC, while the best predictions were made for 70ºC. Regarding polymer proportion, the less accurate predictions were made for 100% carrageenan gels, while 66% carrageenan (34% CMC) displayed the best results. Nevertheless, the R^2 coefficient value suggests that the prediction equation for hardness will give a valid result 96% of the times.

Similar to hardness, adhesiveness prediction had higher residuals for 10% polymer concentration compared to 7.5%. Considering temperature less accurate results were obtained at 30ºC, while the best predictions were made for 60ºC. Concerning polymer proportion, the less accurate prediction were made for 75% carrageenan (25% CMC) gels, while 50% carrageenan (50% CMC) showed the lower residuals.

However, the R^2 coefficient value suggests that the prediction equation will yield a valid result 81% of the times. Equations for predicting hardness and adhesiveness in terms of uncoded variables are shown in equations 1 and 2.

$$\begin{aligned}\log(H) = 1.651 + 0.01947P + 0.0539C - 0.0626T + 0.000125P^2 \\ + 0.000304T^2 + 0.000698PC - 0.000415PT \\ + 0.002223CT\end{aligned} \tag{1}$$

$$\begin{aligned}-log(A^2) = -1.22 + 0.13271P + 0.792C - 0.0256T - 0.000628P^2 \\ + 0.000547T^2 - 0.005018PC - 0.000609PT \\ - 0.006CT\end{aligned} \tag{2}$$

Where H is hardness (N x 10^2), A is adhesiveness (N s x 10^2), T is temperature (°C), P is carrageenan proportion (expressed as carrageenan %), and C is the total polymer concentration (%). Except for the term C^2 that was not significant for both equations, all terms were significant ($p<0.05$) for predicting hardness and adhesiveness.

Predictions obtained by the equations, confirmed that gels hardness increased when temperature dropped and when total polymer concentration increased; harder gels were obtained using higher carrageenan ratios.

On the other hand, adhesiveness showed effects of variables interactions, the property increased until reaching a maximum point and thereafter decreased. It can also be concluded that the more adhesive gels are formed at higher polymer concentrations, lower temperatures and 1:1 proportion of Carr:CMC.

For each gelatin system (30, 40 or 50 %) at 30°C, optimization was carried out for both hardness and adhesiveness. According to the results, the conditions predicted to simulate gelatin systems are found in Table VII. To determine the adequacy of the obtained models, validation tests were performed for each proposed system; measured responses are also reported in Table VII.

As it can be observed in Table VII, there is agreement between the measured values obtained from the experimental and predicted values derived from the models; more so for adhesiveness than for hardness.

It can be seen that hardness as well as adhesiveness of all studied gelatin systems at 30ºC can be obtained using carrageenan and carboxymethyl cellulose at different proportions, concentrations and temperatures. However it is important to evaluate whether the process or product can be adjusted to suit the proposed conditions.

TABLE VII. OPTIMIZATION FACTORS PREDICTED AND MEASURED RESPONSES.

		30% gelatin	40% gelatin	50% gelatin
Factors	Carrageenan %	75.76	78.04	97
	Polymer concentration %	7.5	7.62	7.52
	T (ºC)	47.4	45.9	38.1
Predicted Responses	Hardness (N 10^{2})	165.76	275.98	1153.00
	desirability	0.95648	0.99998	0.98957
	Adhesiveness (N s 10^{2})	-114.12	-128.96	-121.76
	desirability	0.83727	0.99998	0.99443
	Composite desirability	0.8949	0.99998	0.992
Measured Responses	Hardness (N 10^{2})	129.16±11.19	273.02±0.60	1230.8±3.37
	Adhesiveness (N s 10^{2})	-119.68±26.12	-110.99±9.34	-133.10±6.42

Each measured response represents the mean of three measurements ±SD (n=3).

IV. CONCLUSIONS

This study highlighted the mechanical properties of gelatin gels in relation with its concentration and temperature; it can be concluded that hardness, adhesiveness and springiness increased as concentration increased and temperature decreased; there was an important shift in those properties when temperature decreased from 40 to 30ºC. Cohesiveness on the other hand did not show a relation neither with temperature nor with concentration for most cases. CMC gels can be compared with gelatin gels at higher temperatures but the texture profile differs significantly when cooling down. Therefore, the results obtained suggest that mechanical properties of gelatin gels at the studied conditions cannot be obtained using solely one polysaccharide.

Given that there is an important shift in gelatin gels when cooling down from 40 to 30ºC, the optimization results showed that mechanical properties of gelatin gels at different concentrations at 30ºC, could be obtained with different Carr:CMC proportions, concentrations and temperatures. These findings may contribute to the efforts of using polysaccharides as gelatin alternatives to form gels. concentrations and temperatures. These findings may contribute to the efforts of using polysaccharides as gelatin alternatives to form gels.

ACKNOWLEDGMENTS

Author Dominguez-Courtney acknowledges the financial support for her Ph.D. studies in Food Science to CONACyT (Consejo Nacional de Ciencia y Tecnología, Mexico) and Universidad de las Américas Puebla (UDLAP).

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