RHEOLOGICAL CHARACTERISATION OF YOLK-BASED GELS AND *STAPHYLOCOCCUS* GROWTH

Paula Alonso, Amanda Laca*, Adriana Laca, Mario Díaz
Department of Chemical and Environmental Engineering
University of Oviedo. C/ Julián Clavería s/n. 33071 Oviedo. Spain.

Corresponding author: *lacaamanda@uniovi.es

ABSTRACT

Model yolk-based (10% v/v) gels with different concentrations of κ-carrageenan (0 - 2% w/w) were characterised employing rheological measurements, textural analysis and scanning electron microscopy. Additionally, the effect of the microstructure of the model gels on the growth rate of *Staphylococcus* was also evaluated. In all cases, the nature of the gel was dominated by the elastic component, specifically, 1.5, 1.75 and 2% κ-carrageenan samples can be described as "true gels" ($tan\ \delta < 0.1$). Maximum strength of the interactions between rheological units (A) was observed with 1.75% κ-carrageenan (4.74 ± 0.38 kPa), which indicates that the strength of interactions was determined not only by κ-carrageenan concentration, but also by the amount of yolk. Finally, an inverse linear correlation was found between the maximum specific growth rate of *Staphylococcus* and rheological data (R^2 >0.99).

Key words: rheology, κ-carrageenan, egg yolk, food safety, structure; *Staphylococcus*.

1. INTRODUCTION

Many foods exist in the form of gels, which are complex systems with solid-like properties. The rheological characterization of a gel is of great significance and may

help to establish a relation between its constituents and structure (Laca et al., 2011; Basu et al., 2017). In food processing, gelling is an important functional property of polysaccharides that plays a critical role in the characteristics of the final product. κ-carrageenan, a sulphated polysaccharide extracted from various species of red algae, is one of the most widely used gelling agents in the food industry and is also employed as a thickener, stabilizer and emulsifier (Chen et al., 2019). In addition, hen egg is one of the most versatile products, being widely used in the food industry due to its multifunctional properties, especially its natural ability to form gel networks (Zhang et al., 2019).

Staphylococcus aureus has recently been identified as the fifth most common of the pathogens known to cause foodborne illness, being considered as one of the most significant threats to public health (Rubab et al., 2018). *S. aureus* is naturally present as a commensal in the flora of the human nose, skin and mucous membranes. Specifically, it has been reported that 30-50% of the general population are asymptomatic carriers (Alhashimi et al., 2017; Rubab et al., 2018). Therefore, handling food after sniffing, coughing or scratching skin, hair or eyes may cause a contamination of food with *S. aureus* (Geppert et al., 2019; Rebouças et al., 2017).

Food products are composite matrices of multiple constituents and phases, characterised by great structural complexity (Aspridou et al., 2014). In solid or semisolid foods, bacteria are submerged inside the food matrix with limitations on the diffusion of nutrients and metabolites. Hence, a solid environment causes stress to colonies, which may result in changes in metabolism, cell development, morphology, membrane permeability, surface tension and osmotic pressure (Costello et al., 2018; Smet et al., 2015; Verheyen et al., 2018). It seems obvious that, from a safety perspective, knowledge about how food microstructure affects the growth of different

53 microorganisms is a topic of great interest (Aspridou et al., 2014). The influence on
54 microbial kinetics of several aspects of food microstructure has been reported in
55 previous work (Costello et al., 2018; Verheyen et al., 2018; Zilelidou and Skandamis,
56 2019). In most of these studies, however, the food systems were prepared with different
57 microstructures by adding or removing components, such as fat, emulsifiers and/or
58 gelling agents, resulting in noticeable differences in their compositional and
59 physicochemical aspects. The minimisation of these variations between model systems
60 would help to clarify the different microstructural effects on microbial kinetics
61 (Verheyen et al., 2018). The majority of these studies have investigated the growth of
62 *Listeria monocytogenes* (Aryani et al., 2016; Aspriou et al., 2014; Costello et al., 2018;
63 Verheyen et al., 2018; Zilelidou & Skandamis, 2018), although other bacteria, such as
64 *Salmonella* or *E. coli*, have also been evaluated (Smet et al., 2015).
65 In this work, the structure of model yolk gels prepared with different concentrations
66 of κ-carrageenan has been characterised by means of rheological measurements, textural
67 analyses and scanning electron microscopy. In addition, the growth of *Staphylococcus*
68 in the different gels has been monitored to analyse the possible effect of structure on
69 bacterial behaviour. The main novelty of this study is that these food systems have been
70 formulated with minimal variations in their compositional aspects, so the effect of
71 microstructure on growth kinetics has been investigated without being affected by food
72 gel composition. It is also remarkable that, despite being responsible for many
73 foodborne outbreaks, *Staphylococcus* growth has scarcely been investigated in relation
74 to food structure. Particularly, regarding yolk-based foodstuffs, this pathogen has
75 received little attention in the literature in comparison, for example, with *Salmonella*.
76

2. MATERIALS AND METHODS

2.1 Microorganism

Due to its lower associated risk, *S. warneri*, an opportunistic pathogenic species of staphylococcus that has been isolated from eggs, has been employed as a surrogate for *S. aureus* (Neira et al., 2017). Specifically, *Staphylococcus warneri* (CECT 236) acquired from the Spanish Collection of Type Cultures was employed as a model bacterium (Sanchez et al., 2019).

2.2 Culture media and model system preparation

Shell eggs were purchased at a local supermarket and they were employed before their "best-before date". The κ-carrageenan was supplied by Sigma-Aldrich.

The pre-inoculum was prepared in sterile condition from a refrigerated stock on Petri dishes by transferring a loopful of the cultures to 500 mL Erlenmeyer flasks containing 100 mL of egg yolk diluted with distilled water (10% v/v). The pre-inoculum was incubated under aerobic conditions (250 rpm) at 37 ºC for 24 hours. The model gels were prepared with egg yolk at 10% (v/v) and different concentrations of κ-carrageenan (0, 0.75, 1, 1.25, 1.5, 1.75 and 2% (w/w)). A volume of 40 mL of pre-inoculum was added to 400 mL of yolk-κ-carrageenan solution tempered at 45 ºC. Then, the mixture was gently shaken in order to achieve homogeneity and was quickly poured into Falcon tubes (30 mL in each tube), where the egg-yolk gel was finally formed. In all cases, the pH of gels was 6.0-6.5. These concentrations of κ-carrageenan were selected because the amount of carrageenan employed in the food industry for the manufacture of sausages, puddings, ice creams, etc. is usually within the range 0.005% - 3% (Manuhara et al. 2016; Saha & Bhhattachharya, 2010).

The initial concentration of microorganisms in the model food gels was between 10^5 and 10^6 CFU/g and samples were incubated at 37 ºC in static conditions. Experiments were carried out in triplicate.

Sampling was carried out by transferring 1 g of each experimental gel to a stomacher bag containing 9 mL of sterile saline solution; once the sample had been homogenized, serial decimal dilutions of the saline solution were plated at least in triplicate onto Nutrient Broth Agar. Petri dishes were incubated at 30 °C for 48 h before counting. All samples were taken in triplicate.

2.3 Characterization of the structured media

2.3.1. Rheometry

A HAAKE MARS II rotational rheometer (ThermoFisher Scientific) was employed for the rheological measurements, using a serrated plate/plate measuring system (PP35Ti) with a gap of 1 mm. To allow the stresses induced during sample loading to relax, samples were rested for at least 20 min before measurement. Analyses were conducted in this sequence: temperature sweep and then frequency sweep. The temperature sweep was performed from 45 to 25 ºC in 600 s (cooling rate of 2 ºC/min) at a constant shear stress of 1 Pa and at a constant frequency of 2π rad/s. After sample gelation, the frequency sweep was carried out from 0.1 to 500 rad/s at a constant shear stress of 1 Pa and a constant temperature of 25 ºC. Analyses were carried out at least in duplicate. Samples were analysed on the day of preparation.

2.3.2. Texture analysis

Tests were conducted according to Valverde et al. (2016). A TA.XT*Plus* Texture Analyzer (Stable Micro Systems) and a load cell of 5000 g was used. Penetration tests with a penetration distance of 4 mm and a speed of 0.5 mm/min were performed employing a cylindrical probe (SMS P/0.5) to characterise gels that had been previously

gelled in Bloom jars and maintained at 25 ºC for 30 min. In these tests, the maximum force recorded corresponds to the "Bloom strength". At least four samples of each gel were measured.

2.3.3. Scanning electron microscopy SEM

Samples were analysed by scanning electron microscopy (SEM), following the method reported by Laca et al. (2010) with slight modifications.

2.3.4. Statistical analyses

Excel software was employed to carry out a one-way ANOVA with a 95% confidence interval to analyse the data.

3. RESULTS AND DISCUSSION

3.1 Rheological characterisation of model foods

3.1.1 Gelation

κ-carrageenan needs to be heated and then cooled to gel (Diañez et al., 2019). In Figure 1, the gelation curves of the food model gels are shown (t = 0 corresponds to the moment when temperature begin to decrease from 45 to 25 ºC). In the case of samples containing κ-carrageenan, two steps can be easily identified during cooling. In the first step, $G'' > G'$, which indicates the predominance of viscous behaviour. When a certain temperature was reached, however, both moduli rapidly increased and their values stabilised, with $G' > G''$, this second step reflects the behaviour of a solid-like material resembling a network (Tornberg, 2017). The gel point occurs at the time at which G' and G'' cross each other at a given frequency, indicating transition from a liquid-like state (sol) to a solid-like state (gel) (Cordobés et al., 2004; García et al., 2015). So the gelation temperature was obtained in this way from temperature sweeps shown in

Figure 1 and it varied from 34 ºC (0.75% κ-carrageenan) to 44 ºC (2% κ-carrageenan). A good linear correlation can be found between gel point values and concentration of κ-carrageenan; the higher the concentration of κ-carrageenan, the higher the gel point (Figure S1). Additionally, it is noteworthy that elastic moduli values after gelation increased with increasing concentration of κ-carrageenan, which reflects the more elastic character of those gels.

In the sample without κ-carrageenan a transition from sol to gel was not observed (Figure 1). During the entire measurement, viscous moduli were higher than elastic moduli, reflecting the liquid-like state of the sample. This can be explained because, despite the presence of egg yolk, the gelation process was determined by κ-carrageenan. It is important to remember that, whereas κ-carrageenan gels during cooling, egg yolk gels during heating at temperatures higher than 65 ºC (García et al., 2015). In the present study, a trial was carried out, i.e., a solution with 1% of κ-carrageenan and without yolk was analysed by means of a temperature sweep, and a coagulation point of 29.8 ºC was observed (data not shown). This value was lower than that obtained in the model food gel with 1% of κ-carrageenan (35.5 ºC), which is in accordance with the results reported by Chen et al. (2019) and Yang et al. (2018). These authors indicated that the incorporation of different compounds, such as polysaccharides or sucrose, into aqueous κ-carrageenan solution increased the gelation temperature and made the network stronger.

3.1.2 Mechanical properties

The storage modulus (*G'*) represents the elastic response of the material, whereas the loss modulus (*G''*) represents the viscous response. The loss tangent ($\tan \delta = G''/G'$) shows whether the material is closer to an elastic solid ($\tan \delta < 1$) or a viscous fluid ($\tan \delta > 1$). Figure S2 illustrates the evolution of storage and loss moduli in the

linear region (between 0.6 and 60 rad/s), obtained from the frequency sweeps, whereas in Figure S3 the average *tan δ* values of the whole frequency interval studied (0.1 - 500 rad/s) *vs* κ-carrageenan concentrations are represented. As can be seen, all samples containing the polymer showed a more elastic than viscous character (*G' > G''* and *tan δ* < 1) in the linear region of frequency. Other studies found in the literature on gels of κ-carrageenan alone or mixed with other compounds, such as gelatine or yolk, reported that, in general, the behaviour of the *G'* and *G''* moduli was independent of the frequency (Ikeda & Nisinari, 2001; Nuñez-Santiago & Tecante, 2007; Aguilar et al., 2011; Derkach et al., 2015). This mostly agrees with results found here, since in almost all the studied model food gels the frequency does not notably affect the value of the moduli, except for sample with 0% κ-carrageenan, which showed a clear dependence on frequency.

In Figure S4 a linear correlation can be seen between average *G'* values of the whole frequency interval studied (0.1 - 500 rad/s) and κ-carrageenan concentrations. This relationship reflects an increase in gel elasticity with increasing amounts of polysaccharide, which is in accordance with results found in the literature for gels of κ-carrageenan and also of gelatine (Derkach et al. 2015).

In all samples containing κ-carrageenan, since the storage modulus is much larger than the loss modulus, with *tan δ* < 1, the elastic component dominates the rheological behaviour of model food gels at all frequencies studied. When the loss tangent is smaller than 0.1, the system is usually characterized as a "true gel", whereas if it is larger, it is considered a "weak gel" (Aspridou et al., 2014; Diañez et al., 2019; Ikeda & Nishinari, 2001). According to Figure S4, 0.75, 1 and 1.25% κ-carrageenan samples correspond to weak gels and 1.5, 1.75 and 2% κ-carrageenan samples can be described as true gels. This decrease in *tan δ* values with the increase in κ-carrageenan

concentrations seen here has previously been reported for egg yolk/κ-carrageenan gel systems (Aguilar et al., 2011).

Gabriele et al. (2001) proposed a model to describe the rheological properties of food gels. So, in order to obtain more detailed information about the mechanical properties of the model food gels employed in this work, the linear region of frequency sweep tests data (0.6-60 rad/s) were correlated to the following power law equation:

$$G^* = A \cdot \nu^{1/z}$$

where G^* is the complex modulus (Pa), ν the frequency (Hz), z (dimensionless) the coordination number and A (G^* in Pa at 1 Hz) the proportional coefficient.

A may be interpreted as the interaction force between the flow units (gel strength) and z is equivalent to the number of flow units interacting with one another. The fitting values obtained for parameters A and z are listed in Table 1. According to ANOVA results there were statistically significant differences between the means of A and z (95% confidence interval). In general, A increased with κ-carrageenan concentration, which reflected that interactions were stronger for higher amounts of polymer. However, all samples containing κ-carrageenan showed values of z in the same order of magnitude without any particular tendency. The maximum value for parameter A was observed with 1.75% κ-carrageenan. This seems to indicate that at this concentration an equilibrium was achieved between yolk and κ-carrageenan interactions. As additional experiments, the A and z parameters were obtained for a sample of 1% κ-carrageenan gel without yolk and values were much lower (A = 0.060 ± 0.012 kPa y z = 9.52 ± 1.60) than those obtained for the gel with the same concentration of κ-carrageenan that contained 10% of yolk. Thus, it is clear that the presence of yolk increases the number of interactions and also the strength of these interactions. This is

in accordance with coagulation curves, which revealed a complex synergism during gel formation between yolk and κ-carrageenan molecules.

3.2 Textural properties of model foods

"Bloom" strength is one of the fundamental functional properties of gelatin and, in general, of gels. The "Bloom" test determines the weight in grams needed for a specified plunger to depress the surface of the gel without breaking it (Schrieber & Gareis, 2007). In Table 1, values obtained from the texture analysis are shown and according to the ANOVA results there were statistically significant differences between the means of "Bloom" strength values (95% confidence interval). A clear trend can be seen, namely a rise in κ-carrageenan percentage producing an increase in "Bloom" strength. Model food gels with lower "Bloom" values have weaker gel strengths, which is corroborated with values found for *tan δ* (Figure S6) and also with parameter *A* values (Table 2). These results are in agreement with those reported by Chen et al. (2017) for gelatins. The higher the "Bloom" value of a gel, the higher the gelling point and the shorter its gelling time (Schrieber & Gareis, 2007), which is in accordance with results obtained from temperature sweeps (Figure 1).

3.3 Microstructure of model foods

In Figure S5, microphotographs of 1, 1.25, 1.75 and 1.75% κ-carrageenan model food gels are shown as an example. In all cases, a fibrous structure that corresponds with the carrageenan scaffolding where globular yolk proteins are integrated can be observed (Laca et al., 2010; Valverde et al., 2017). Microorganisms, which show their characteristic spherical shape (cocci), form grape-like clusters packed in this complex matrix (see black arrows in Figure S7). Chen et al. (2017) indicated that gelatins with higher "Bloom" values did not have voids and showed much smoother, more compact

surface microstructures than samples with lower "Bloom" values, which agrees with the results found here for yolk-carrageenan gels (Table 1).

3.4 Effect of gel structure on *Staphylococcus* growth

In Figure 2, the growth of *S. warneri* in different media is shown (t = 0 corresponds to the sample taken once the medium has been inoculated and is gelled). It can be seen that a lag phase is not observed in any case. This is due to the fact that the pre-inoculum has been grown in a medium with the same composition as the model gels, i.e., egg yolk.

In Table 2 the maximum specific growth rates obtained from the different model gels are summarised. It is usually assumed that the confinement of the bacteria inside a structured medium decreases the cell growth rate. Diffusional limitations represent the main causal mechanism of this phenomenon (Noriega et al., 2008; Noriega et al., 2010a; Noriega et al., 2010b). In this sense, Hooijmans et al. (1990) reported growth rates of *E. coli* of 0.24 h^{-1} in carrageenan gels and 0.30 h^{-1} in free-cell suspensions. On the contrary, here, although μ_{max} values were in the same order of magnitude, the lowest specific growth rate was obtained in liquid medium (0% of κ-carrageenan).

Baka et al. (2016) found that the growth of *L. monocytogenes* was faster in frankfurter sausages when their structure was firmer. These findings indicate that the microstructural effect is complex, and it depends on the compositional (i.e., fat presence) and physicochemical factors of the specific food system. In this study, the highest μ_{max} value was found in the matrix with 0.75% of κ-carrageenan and then the specific growth rates decreased with increasing concentrations of κ-carrageenan. So, *S. warneri* exhibited a particular behaviour, i.e., the presence of κ-carrageenan favoured the growth of the bacterium; however, the growth of the microorganism is faster with

lower concentrations of the polymer. This can be explained by two different effects. Firstly, the incubation was carried out in static conditions, so in the case of the liquid medium, egg yolk lipids form a layer on the surface of the medium, which may impede the diffusion of oxygen. When κ-carrageenan is added to the medium, the mixture is gently shaken to homogenize the matrix, and this homogenization favours the diffusion of the oxygen, which is retained in voids in the network formed by polymer gelation (Figure S5). Secondly, it is well known that diffusional limitations, which can restrict the availability of oxygen and nutrients to immersed colonies, are present in gelled systems (Baka et al., 2017; Costello et la., 2018). The composition of the specific food system can also affect cell development (Baka et al., 2016). Nevertheless, in the present study, the composition is always the same in all the systems analysed, only the amount of κ-carrageenan is modified and κ-carrageenan is not degraded by *S. warneri* as this bacterium does not produce carrageenases (Chauhan & Saxena, 2016).

Aspridou et al. (2014) analysed the growth of *Listeria monocytogenes* in gels formed from different concentrations of sodium alginate and gelatin and found that the μ_{max} was inversely correlated with the storage modulus. In the same way, therefore, the relationship between maximum specific growth rate and structural parameters has been explored here. A good inverse linear correlation was found between *G'* and μ_{max} and the same tendency was observed for "Bloom" strength and μ_{max} (Figure S6). These results agree with results reported by Aspridou et al. (2014) and confirm that it is possible to employ structural parameters to describe food matrix effects on microbial growth kinetics, not only for *Listeria*, but also for other kinds of bacteria such as *Staphylococcus*.

Any increase in the κ-carrageenan concentration resulted in a reduction in growth rate. So, the matrix of the structured medium seems to exert a negative effect on

303 the microorganism, producing a decrease in the growth rate when the bacterium was in
304 the exponential growth phase. This stress may be caused by many different factors.
305 Diffusion limitations, not only of oxygen, but also of nutrients and metabolites, have
306 been reported to take place within different gel type foods (Noriega et al., 2008; Noriega
307 et al., 2010a; Noriega et al., 2010b). As a consequence, concentration gradients of
308 nutrients, metabolites, pH and oxygen may develop around the colony (Aspridou et al.,
309 2014). Temperature, food composition, the presence of fat droplets, the target
310 microorganism and physicochemical properties are also important parameters to be
311 considered (Verheyen et al., 2018). Additionally, the solid matrix may cause changes in
312 metabolism, cell development, morphology, membrane permeability, surface tension
313 and osmotic pressure, parameters that affect the physiological state of the cell (Smet et
314 al., 2015).
315
316 **4. CONCLUSIONS**
317
318 Six model yolk-based gels with the same nutritional composition and with
319 different κ-carrageenan concentrations have been characterised from a structural point
320 of view. According to rheological measurements, the nature of the gel was in all cases
321 dominated by the elastic component and, in addition, in general, the network strength
322 (indicated by parameter *A*) increased with increasing concentrations of κ-carrageenan.
323 These gels have been employed as model systems to evaluate the effect of food
324 microstructure on *Staphylococcus* growth kinetics. It was observed that in the structured
325 media prepared with κ-carrageenan, microorganism growth was faster for lower
326 concentrations of the polymer. Nevertheless, the presence of κ-carrageenan favoured the
327 growth of the bacterium in comparison with the liquid medium.

Good inverse linear correlations between the storage modulus (*G'*) and maximum specific growth rates (μ_{max}) and also between the "Bloom" strength and μ_{max} values have been found, which evidences the utility of structural parameters not only for analysing the quality of food products, but also to evaluate their tendency to be spoiled and to became a health risk due to the dependence of bacterial growth on the food's microstructure.

AKNOWLEDGEMENTS

This study was conducted thanks to funding from the Employment, Industry and Tourism Office of the Principality of Asturias (Spain) through project IDI/2018/000127.

REFERENCES

Aguilar JM, Batista AP, Nunes MC, Cordobés F, Raymundo A, Guerrero A (2011) From egg yolk/κ-carrageenan dispersions to gel systems: Linear viscoelasticity and texture analysis. Food Hydrocolloid 25:654-658.

Aguilar JM, Cordobés F, Raymundo A, Guerrero A (2017) Thermal gelation of mixed egg yolk/kappa-carrageenan dispersions. Carbohydr Polym 161:172-180.

Al-zoreky NS, Al-Taher AY (2019) In vitro and in situ inhibition of some food-borne pathogens by essential oils from date palm (*Phoenix dactylifera* L.) spathe. Int J Food Microbiol 299:64-70.

Alhashimi HMM, Ahmed MM, Mustafa JM (2017) Nasal carriage of enterotoxigenic *Staphylococcus aureus* among food handlers in Kerbala city. Karbala International Journal of Modern Science 3:69-74.

Aryani DC, Zwietering MH, den Besten HMW (2016) The effect of different matrices on the growth kinetics and heat resistance of *Listeria monocytogenes* and *Lactobacillus plantarum*. Int J Food Microbiol 238:326-337.

Aspridou Z, Moschakis T, Biliaderis CG, Koutsoumanis KP (2014) Effect of the substrate's microstructure on the growth of *Listeria monocytogene*s. Food Res Int 64:683-691.

Baka M, Noriega E, Van Langendonck K, Van Impe JF (2016) Influence of food intrinsic complexity on *Listeria monocytogenes* growth in/on vacuum-packed model systems at suboptimal temperatures. Int J Food Microbiol 235:17-27.

Basu S, Shivhare US, Chakraborty P (2017) Influence of sugar substitute in rheology of fruit gel. In: Ahmed J (ed.) Advances in food rheology and its applications. Woodhead Publishing Series in Food Science, Technology and Nutrition, pp. 335-376. Elsevier Inc.

Chauhan PS, Saxena A (2016) Bacterial carrageenases: an overview of production and biotechnological applications. 3 Biotech 6:146.

Chen M, Liu F, Chiou B-S, Sharif HR, Xu J, Zhong F (2017) Characterization of film-forming solutions and films incorporating free and nanoencapsulated tea polyphenol prepared by gelatins with different Bloom values. Food Hydrocolloid 72:381-388.

Chen J, Chen W, Duan F, Tang Q, Li X, Zeng L, Zhang J, Xing Z, Dong Y, Jia L, Gao H (2019) The synergistic gelation of okra polysaccharides with kappa-carrageenan and its influence on gel rheology, texture behaviour and microstructures. Food Hydrocolloid 87:425-435.

Cordobés F, Partal P, Guerrero A (2004) Rheology and microstructure of heat induced egg yolk gels. Rheol Acta 43:184-195.

Costello KM, Gutierrez-Merino J, Bussemaker M, Ramaioli M, Baka M, Van Impe JF, Velliou EG (2018) Modelling the microbial dynamics and antimicrobial resistance development of *Listeria* in viscoelastic food model systems of various structural complexities. Int J Food Microbiol 286:15-30.

Dianez I., Gallegos C, Brito-de la Fuente E, Martínez I, Valencia C, Sánchez, MC, Díaz MJ, Franco JM (2019) 3D printing in situ gelification of κ-carrageenan solutions: Effect of printing variables on the rheological response. Food Hydrocolloid 87:321-330.

Derkach SR, Ilyin SO, Maklakova AA, Kulichikhin VG, Malkin AY (2015) The rheology of gelatin hydrogels modified by κ-carrageenan. LWT - Food Sci. Technol 63: 612-619.

Gabriele D, de Cindio B, D'Antona P (2001) A weak gel model for foods. Rheol Acta 40:120-127.

García V, Laca A, Paredes B, Martínez LA, Rendueles M, Díaz M (2015) Development and characterization of a new sweet egg-based dessert formulation. Int J Gastron Food Sci 2:72-82.

Geppert J, Schulze Struchtrup S, Stamminger R, Haarhoff C, Ebert V, Koch S, Lohmann M, Böl G-F (2019) Food safety behavior observed in German TV cooking shows. Food Control 96:205-211.

Hooijmans CM, Briasco CA, Huang J, Geraats BGM, Barbotin, JN, Thomas D, Luyben KChAM (1990) Measurement of oxygen concentration gradients in gel-immobilized recombinant *Escherichia coli*. Appl Microbiol Biotechnol 33:611-618.

Ikeda S, Nishinari K (2001) "Weak gel"-type rheological properties of aqueous dispersions of nonaggregated κ-carrageenan helices. J Agr Food Chem 49:4436-4441.

Laca A, Paredes B, Díaz M (2010) A method of egg yolk fractionation. Characterization of fractions. Food Hydrocolloid. 24, 434-443.

Laca A, Paredes B, Díaz M (2011) Thermal behaviour of lyophilized egg yolk and egg yolk fractions. J Food Eng 102:77-86.

Manuhara GJ, Praseptiangga D, Riyanto RA (2016) Extraction and characterization of refined k-carrageenan of red algae [Kappaphycus alvarezii (Doty ex P.C. Silva, 1996)] Originated from Karimun Jawa Islands. Aquatic Procedia. 7:106-111.

Neira C, Laca A, Laca A, Díaz M (2019) Microbial diversity on commercial eggs as affected by the production system. A first approach using PGM. Int J Food Microbiol 262:3-7.

Noriega E, Laca A, Díaz M (2008) Modelling of diffusion-limited growth for food safety in simulated cheeses. Food and Bioprod Process 86:122-129.

Noriega E, Laca A, Díaz M (2010a) Development of a structure-based model for the competitive growth of *Listeria innocua* in minced chicken breasts. Int J Food Microbiol 142:44-52.

Noriega E, Laca A, Díaz M (2010b) Decisive role of structure in food microbial colonization and implications for predictive microbiology. J Food Prot 73:938-951.

Núñez-Santiago MC, Tecnate A (2007) Rheological and calorimetric study of the sol-gel transition of κ-carrageenan. Carbohydr Polym 69:763-773.

Rebouças LT, Santiago LB, Martins LS, Rios Menezes AC, Araújo MDPN, Almeida RCDC (2017) Food safety knowledge and practices of food handlers, head chefs and managers in hotels' restaurants of Salvador, Brazil. Food Control 73:372-381.

Rubab M, Shahbaz HM, Olaimat AN, Oh D-H (2018). Biosensors for rapid and sensitive detection of *Staphylococcus aureus* in food. Biosens Bioelectron 105:49-57.

Saha D, Bhattacharya S (2010) Hydrocolloids as thickening and gelling agents in food: A critical review. Journal of Food Science and Technology 47:587-597.

Sanchez, M., Neira, C., Laca, A., Laca, A., Díaz, M. (2019) Survival and development of *Staphylococcus* in egg products. LWT - Food Sci. Technol 101:685-693.

Schrieber R, Gareis H (2007) Gelatine Handbook: Theory and Industrial Practice. Wiley-VCH Verlag GmbH & Co. KGaA.

Smet C, Van Derlinden E, Mertens ., Norieg, ., Van Imp, JF (2015) Effect of cell immobilization on the growth dynamics of *Salmonella* Typhimurium and *Escherichia coli* at suboptimal temperatures. Int J Food Microbiol 208:75-83.

Tornberg E (2017) Influence of fibers and particle size distribution on food rheology. In: Ahmed J (ed) Advances in food rheology and its applications. Woodhead Publishing Series in Food Science, Technology and Nutrition, pp. 177-208. Elsevier Inc.

Valverde D, Laca A, Estrada LN, Paredes B, Rendueles M, Díaz M (2016) Egg yolk and egg yolk fractions as key ingredient for the development of a new type of gels. Int J Gastron Food Sci 3:30-37.

Verheyen D, Bolívar A, Pérez-Rodríguez F, Baka M, Skåra T, Van Impe JF (2018) Effect of food microstructure on growth dynamics of *Listeria monocytogenes* in fish-based model systems. Int J Food Microbiol 283:7-13.

Yang Z, Yang H, Yang H (2018) Effects of sucrose addition on the rheology and microstructure of κ-carrageenan gel. Food Hydrocolloid 75:164-173.

Zhang M, Li J, Chang C, Wang C, Li X, Su Y, Yang Y (2019) Effect of egg yolk on the textural, rheology and structural properties of egg gels. J Food Eng 246: 1-6.

448 Zilelidou EA, Skandamis PN (2018) Growth, detection and virulence of *Listeria*
449 *monocytogenes* in the presence of other microorganisms: microbial interactions from
450 species to strain level. Int J Food Microbiol 277:10-25.
451

Table 1. Power-law parameters (*A* and *z*) obtained from frequency sweeps (in all cases $R^2 > 0.90$) and "Bloom" strength of model gel foods. Average values ± SD are shown.

κ-carrageenan (%)	*A* (kPa)	*z* (-)	"Bloom" strength (g)
0	0.0005 ± 0.00001	0.43 ± 0.02	-
0.75	0.41 ± 0.01	28.85 ± 3.39	17.5 ± 1.5
1	0.87 ± 0.09	45.08 ± 5.56	14.1 ± 0.4
1.25	2.27 ± 0.04	36.38 ± 13.12	52.9 ± 11.7
1.5	3.27 ± 0.02	32.42 ± 3.76	93.9 ± 13.5
1.75	4.74 ± 0.38	27.58 ± 2.83	103.8 ± 19.1
2	4.18 ± 0.45	33.17 ± 6.04	163.2 ± 11.5

Table 2. Maximum specific growth rate (μ_{max}) values obtained with different concentrations of κ-carrageenan. In all cases $R^2 \geq 0.90$.

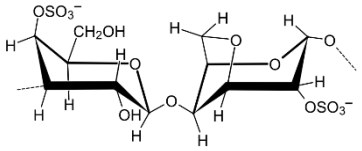
κ-carrageenan (%)	μ_{max} (h^{-1})
0	0.1320
0.75	0.1712
1	0.1669
1.25	0.1601
1.5	0.1519
1.75	0.1501
2	0.1440

FIGURE CAPTIONS

Figure 1. (A) Gelation curve of 0% κ-carrageenan sample and gelation curve of model food gels containing κ-carrageenan: 0.75% (B), 1% (C), 1.25% (D), 1.5% (E), 1.75% (F) and 2% (G). *G'* (orange), *G''* (blue) and temperature (black). Average values are represented.

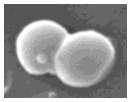
Figure 2. Growth of *S. warneri* in media with different concentrations of κ-carrageenan: 0% (black), 0.75% (light blue), 1% (red), 1.25% (green), 1.5% (dark blue), 1.75% (orange) and 2% (violet). Data from triplicates are represented and, in all cases relative standard deviation < 5%. Discontinuous lines correspond with the first-order fitting to data of the exponential phase to obtain μ_{max} values shown in Table 2.

κ-carrageenan

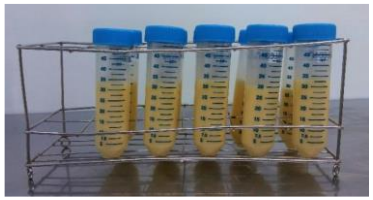


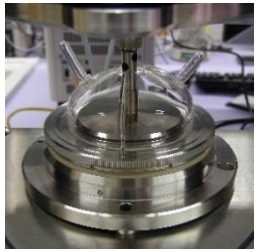
Egg yolk

Staphylococcus

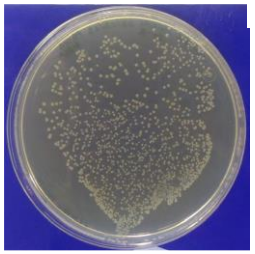


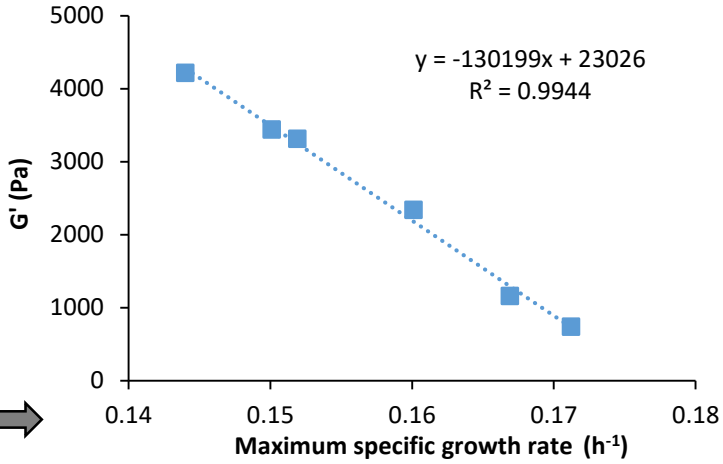
Model food gels

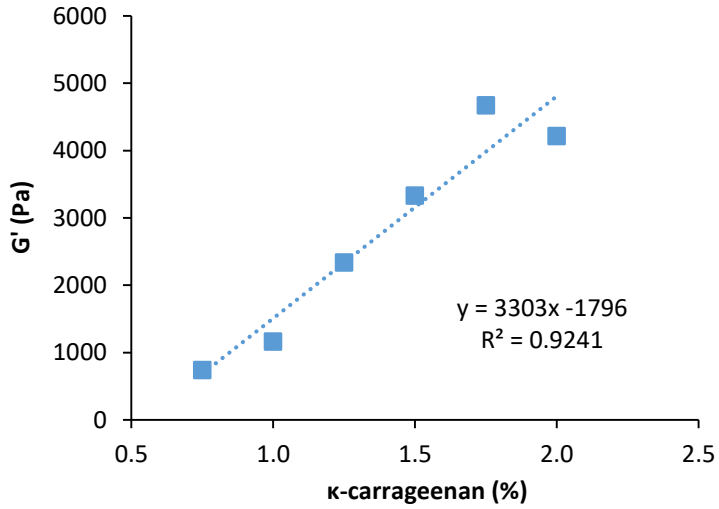




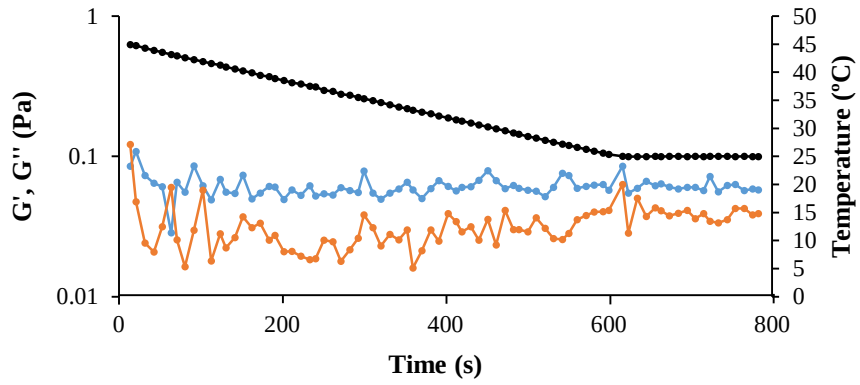
Structure and microorganism growth analysis



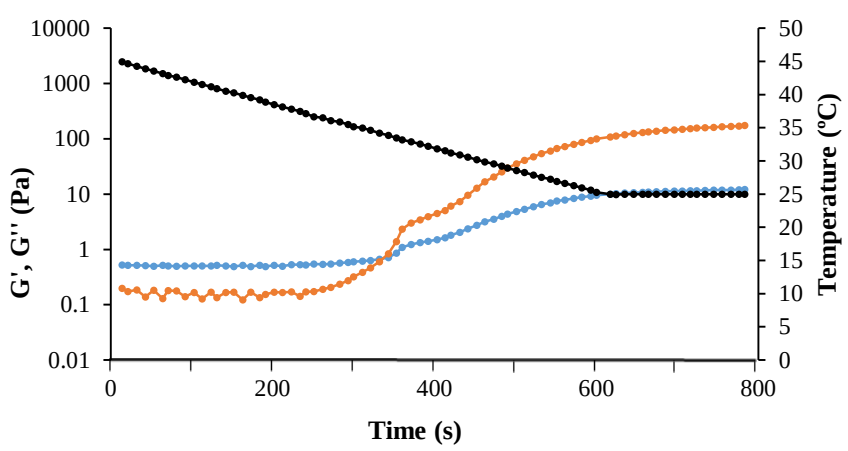




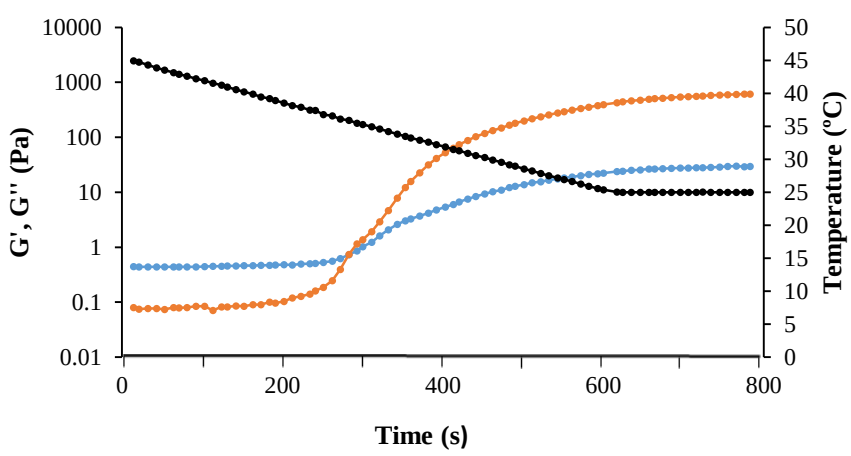
A)


G', G'' (Pa)
Temperature (ºC)
Time (s)

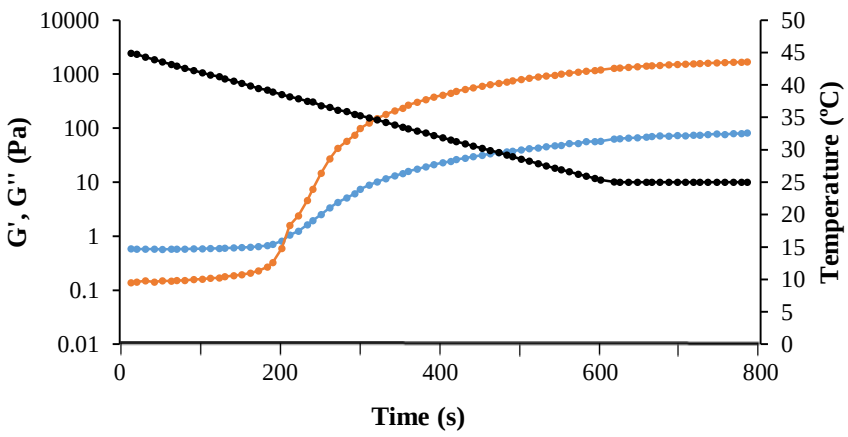
B)


G', G'' (Pa)
Temperature (ºC)
Time (s)

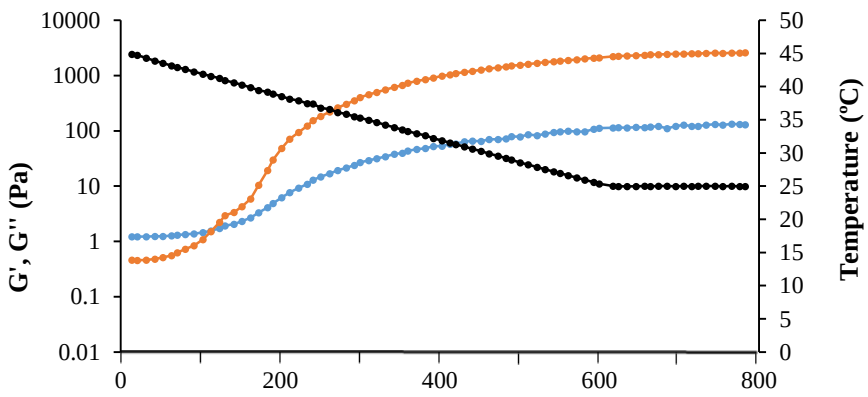
C)


G', G'' (Pa)
Temperature (ºC)
Time (s)

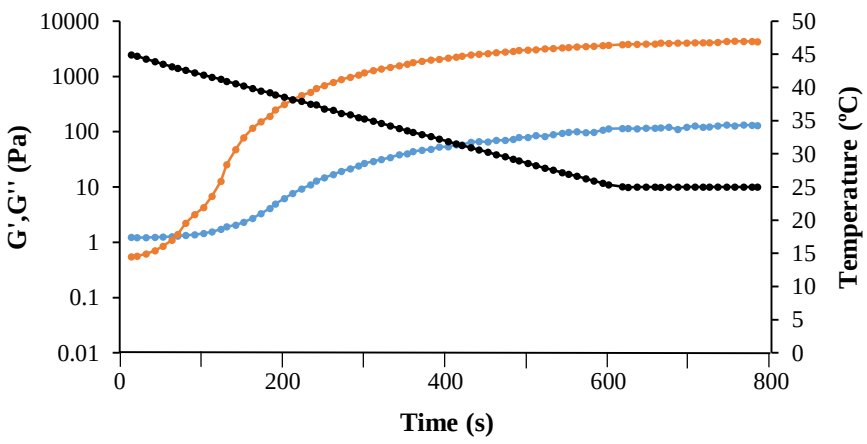
D)


G', G'' (Pa)
Temperature (ºC)
Time (s)

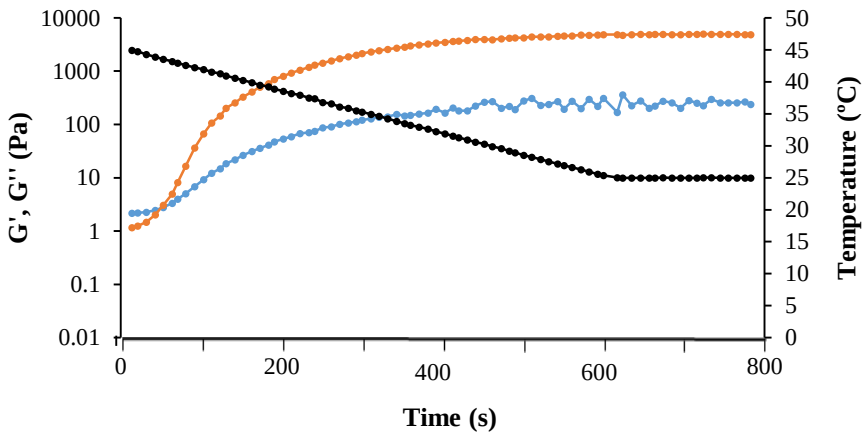
E)

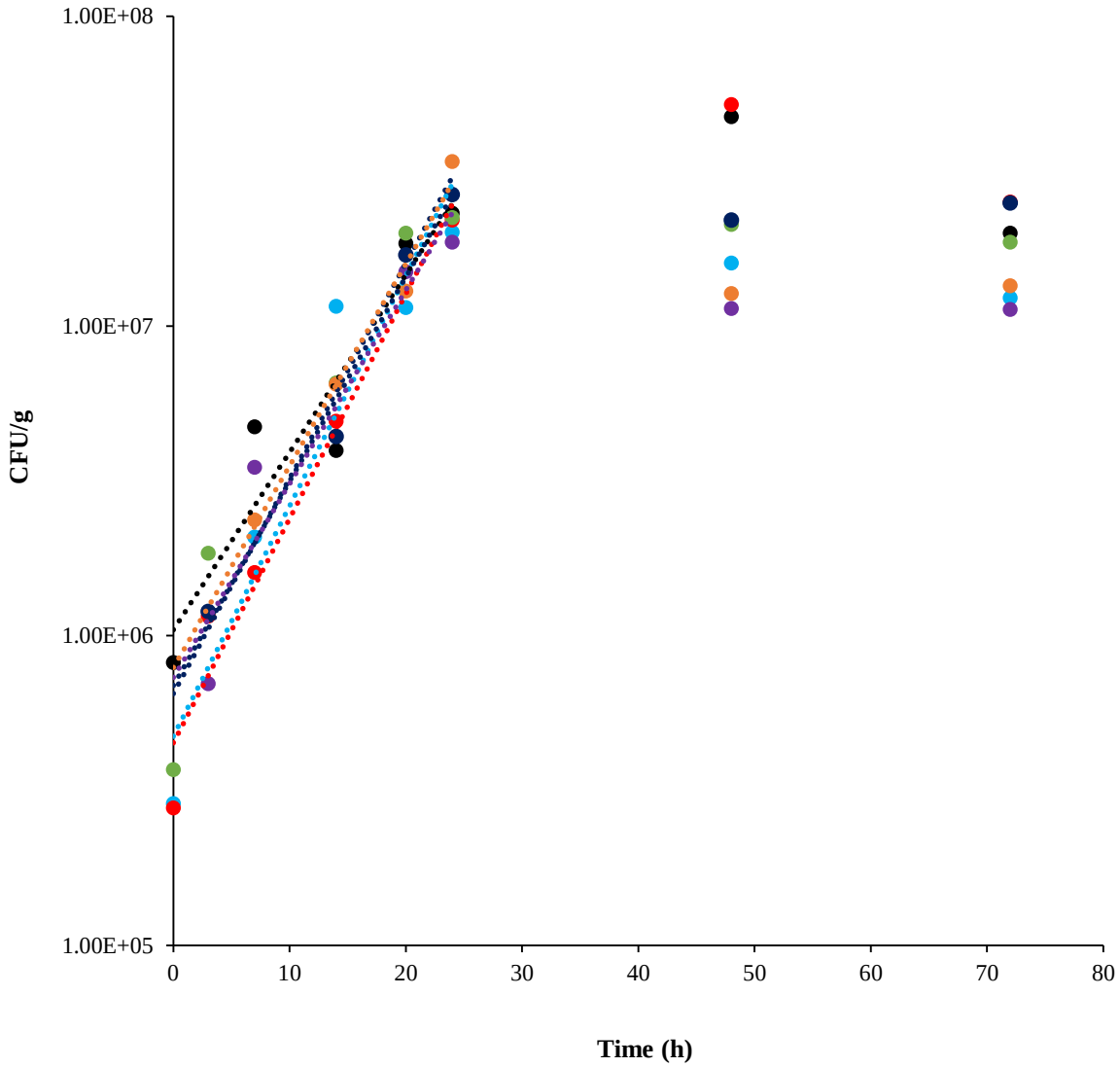

G', G'' (Pa)
Temperature (ºC)

F)


G',G'' (Pa)
Temperature (ºC)
Time (s)

G)


G', G'' (Pa)
Temperature (ºC)
Time (s)


1.00E+08
1.00E+07
1.00E+06
1.00E+05
CFU/g
0
10
20
30
40
50
60
70
80
Time (h)