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Influence of mixed gel structuring with different degrees of matrix inhomogeneity on oral residence time

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Abstract

The aim of this study was to examine the influence of structuring mixed biopolymer gels with different degrees of inhomogeneity on oral residence time. Ten model gels with varying mechanical and structural properties were prepared using *κ*-carrageenan and sodium alginate at concentrations ranging from 0-4 wt%. In few of the mixed gel systems, structural inhomogeneity was introduced by incorporation of calcium alginate beads of different sizes, later made by syringe extrusion or spraying techniques. The gels were characterized by dynamic oscillation, fracture behaviour and the structural details were evidenced in different length scales by cryo-scanning electron microscopy (cryo-SEM) and transmission electron microscopy (TEM). In parallel, gels were characterized by quantitative descriptive analysis (QDA™). Oral processing behaviour was assessed in terms of oral residence time, number of chews and difficulty perceived by eleven young participants. A decrease in the gel fracture point with the addition of calcium alginate beads was attributed to the interruption of the continuous *κ*-carrageenan gel network, as revealed in the Cryo-SEM and TEM images and with narrower linear viscoelastic region. When the mixed gel network included *κ*-carrageenan with sodium alginate, the linear viscoelastic range was extended, but the gel strength was lower than *κ*-carrageenan alone highlighting the incompatibility between the biopolymers. Oral residence time was highly dependent on the number of chews and to a certain extent on the difficulty perceived. Oral residence time and number of chews were positively correlated with gel strength, degree of network inhomogeneity in terms of particle size of the beads.

Key words: gel inhomogeneity, particles, mixed gels, oral residence time, swallowing, structure

1. Introduction

Swallowing is a vital part of oral processing, it is a complex act that involves functional coordination of the mouth, pharynx, larynx and oesophagus (Palmer, Drennan, & Baba, 2000). Swallowing disorders may occur due to functional as well as physiological inabilities (Matsuo & Palmer, 2008). Chronic swallowing disorders such as dysphagia are common among the elderly population (Roy, Stemple, Merrill, & Thomas, 2007). They are associated with different pathological conditions, such as Parkinson's disease, Alzheimer's disease, dementia, throat cancer (Ekberg, Hamdy, Woisard, Wuttge–Hannig, & Ortega, 2002; Kumlien & Axelsson, 2002), or with the natural body age-linked degeneration, that tend to increase the risk of aspiration and thus, pneumonia (Nishikubo, et al., 2015). In the elderly population, swallowing disorders may lead to malnutrition, which is a severe geriatric syndrome related to risk of infections, impaired recovery and mortality (Norman, Pichard, Lochs, & Pirlich, 2008).

Clinical researchers have approached swallowing disorders by studying anatomic structures and flow of the food bolus (Palmer et al. 2000) through videofluoroscopy (Langmore, 2003; Palmer, et al., 2000), fiberoptic endoscopic (Dua, Ren, Bardan, Xie, & Shaker, 1997) or ultrasound equipment (Koshino, Hirai, Ishijima, & Ikeda, 1997) among others. In parallel, food scientists have investigated the role of precise optimization of viscosity of food biopolymers with an objective of manipulating the swallowing process. One of the main conclusions of previous researches is that increasing viscosity of food and thereby increased oral residence time is an effective strategy to combat swallowing disorders (aspiration) (Logemann, 2007). Hydrocolloids have been commonly used as thickeners in food for swallowing disordered and/or dysphagia patients (Zargaraan, Rastmanesh, Fadavi, Zayeri, & Mohammadifar, 2013) these thickeners were conventionally xanthan gum or starch based

(Leonard, White, McKenzie, & Belafsky, 2014; Seo & Yoo, 2013). Garcia, Chambers, Matta, & Clark (2005) concluded that in comparison to a thin bolus, a thicker bolus will be residing in mouth for a relatively longer time. This sensory feedback of slow bolus flow through the oropharynx will protect airways (Nicosia & Robbins, 2007). Thickened diets have shown to improve the nutritional status of the patients as well as their hydration level due to lower chances of aspiration and pneumonia (Rofes, et al., 2010).

Using hydrocolloid based test fluids and gels have been effective model systems to study the influence of rheological properties in the food oral processing (Hayakawa, et al., 2014; Hori, et al., 2015; Ishihara, Nakauma, Funami, Odake, & Nishinari, 2011; Kohyama, et al., 2015; Moritaka & Nakazawa, 2010), with the aim to design food for people suffering from dysphagia and/or at risks of swallowing disorders. Other advantage of working with model hydrocolloids is that they are not emotionally linked and excludes the postprandial satisfaction and flavour experience, which occurs when testing with well-known real food-products (Prescott, 2012; Yeomans, 2012). It was found that the difficulty associated with an increment of time in the mouth (Moritaka, et al., 2010) was linked with sensory attributes such as resistance to fracture (Hayakawa, et al., 2014; Laguna, Barrowclough, Chen, & Sarkar, 2016), which further highlights the influence of viscosity and/or gel strength of the liquid or semi-solid food on oral processing.

Although the influence of consistency on time in mouth is well researched, there has been scant literature on the complex interplay between structural properties of gels and oral processing. In this study, we hypothesize that not only viscosity but also the degree of structure can increase time in mouth. Hence, this study aims to explore different factors to increase the time in mouth: the gel strength, structural complexity, or the interaction between

gel strength and complexity. To achieve this objective, we have created a series of edible *κ*-carrageenan gels without or with sodium alginate or inclusion of calcium alginate beads with diverse mechanical and oral processing properties via precise manipulation of structural inhomogeneity (i.e. different concentrations and particle size of the beads).

κ-Carrageenan is a biopolymer with repeating disaccharide units of 3-linked *β*-d-galactose 4-sulfate and 4-linked 3,6-anhydro-*α*-d-galactose. *κ*-Carrageenan can form thermo-reversible gels at low concentrations and the gelation involves coil-to-helix molecular transition of the *κ*-carrageenan molecules followed by aggregation that occur upon cooling (Morris, Rees, & Robinson, 1980). Sodium alginate is a linear anionic polysaccharide derived from brown seaweeds, consisting of *β*-1,4-D-mannuronic acid (M-block) and *α*-1,4-L-glucuronic acid (G-block). Sodium alginate can undergo ionic crosslinking upon contact with calcium ions in aqueous solution to form an "egg-box model" gel structure (Yoo, Song, Chang, & Lee, 2006). The divalent calcium displaces the sodium ion and due to the physical crosslinking or chelation between the carboxylate anions of guluronate units in alginate and the calcium ions, the calcium-alginate gel beads are formed. Mixing of these distinct macromolecules may result in the formation of two microscopic layers, with each containing most of one constituent and little of the other. The phenomenon is known as phase separation and, in the gel state, the phase morphology of the mixture determines the overall structure (Goh, Sarkar, & Singh, 2008, 2014) and thus may have an influence on the oral processing behaviour. Furthermore, incorporation of food-grade calcium alginate beads in a *κ*-carrageenan "continuous" biopolymer matrix may increase/ decrease the mechanical strength of the mixture depending upon the interaction, which might further influence the oral residence time.

To our knowledge, this is the first study that generates insights on impact of mixed gel structuring on oral residence time by employing a holistic combination of characterization of these mixed gels using structural, mechanical (small and large deformation rheology), sensory and oral processing techniques.

2. Materials and methods

2.1. Sample preparation

κ-Carrageenan and sodium alginate were both obtained from Special Ingredients (Sheffield, UK). Calcium chloride was obtained from Mineral Water (Purfleet, UK). All three ingredients were food grade and used without any further purification. The concentration of the biopolymers is summarized in Table 1.

Calcium alginate beads production (CAl). Firstly, sodium alginate solutions were prepared by slowly adding the exact quantity of the powder in distilled water. The obtained dispersion were then heated and stirred for 1 h at 90 °C to ensure complete solubilisation. Calcium chloride solutions (2M) were prepared by dissolving the required quantity in distilled water. For the preparation of big beads, sodium alginate (Na alginate) solution was extruded using a 0.8 mm nozzle syringe (Terulo, Neolus) into the calcium chloride bath. For the small beads, sodium alginate solution was sprayed at 50-55 mL/min over the calcium chloride bath using jet sprayer (0.45 mm nozzle diameter). The Na-alginate beads were cross-linked by Ca^{2+} ions to form sprayed Ca-alginate beads. Both beads (big and small i.e. sprayed, particle size is summarized in Table 2) remained in the $CaCl_2$ bath for 30 minutes; the prepared beads were removed and washed with deionized water twice to remove any non-cross-linked Ca^{2+} ions.

149 *κ*-Carrageenan gel production (*κ*). 1-4 wt% of *κ*-carrageenan (as indicated in Table 1) was
150 prepared by dissolving appropriate quantities of *κ*-carrageenan in distilled water and mixed
151 by magnetic stirring for a few hours at 80 °C to facilitate hydration.
152
153 *κ*-Carrageenan and sodium alginate gel production (M-*κ*SAl). Binary gel preparation
154 involved dry blending of appropriate quantities of *κ*-carrageenan and sodium alginate and
155 dissolving in distilled water (1.0 and 2.0 wt%) followed by magnetic stirring for a few hours
156 at 80 °C.
157
158 *κ*-Carrageenan and calcium alginate bead production (B-*κ*CAl/ S-*κ*CAl). Small (spray) or big
159 beads were added to tray (12×7.5×1.5 cm length, width, depth), then, *κ*-carrageenan solution
160 of 1-2 wt% concentration (80 °C) was poured in to the tray in 1:1 w/w. After storage at 4 °C
161 for 24 h, gels were cut in a circular shape (2.0×1.0 cm; diameter × height).
162
163 **2.2. Rheological measurements**
164 2.2.1 Small deformation rheology
165 The rheological properties of the mixed gels were analysed by dynamic oscillatory
166 measurement in a Kinexus rheometer (Malvern, UK). Gel cylinder of 30 mm diameter were
167 placed into a pre-heated plate (37 °C), the rheometer was equipped with a 30 mm of parallel
168 plate. Considering that the gap between the plates should be larger than the biggest bead, a
169 gap of 3 mm was selected. A strain sweep test from 0.01-100% was carried out to determine
170 the linear viscoelastic region at constant angular frequency of 1 Hz. Frequency sweeps were
171 conducted from 0.01-100 Hz at constant strain of 0.05%. The elastic (storage modulus, G′)
172 and viscous modulus (loss modulus, G″) and complex moduli (G*) were recorded.
173 Experiments were replicated three times.

174 2.2.2. Large deformation rheology

175 To characterize the mechanical properties, fracture mechanics of mixed gels were conducted
176 by both penetration test using upper Volodkevich Bite Jaw and compression test using 75-
177 mm diameter aluminium plate (P/75) (Texture analyser, Stable Micro Systems, Godalming,
178 UK). Since human frontal teeth are around 8-9 mm, Volodkevich probe of 10 mm was
179 considered for simulating the human dents (Brandão & Brandão, 2013; Gillen, Schwartz,
180 Hilton, & Evans, 1994). Each test was performed for five times for each sample, placing the
181 sample on a flat platform at a room controlled temperature of 25°C. In the penetration test,
182 the controlled speed of the probe was 1.0 mm per second for 5.0 mm of penetration; in the
183 compression test, the probe was at 5mm per second of controlled speed at 50% of strain. The
184 maximum force (N) as a measure of hardness, the number of force peaks (with a threshold of
185 0.1 N) as an index of gel break layers and the gradient of the initial steep slope of the curve
186 (N/sec) as a measure of gel deformation were assessed. Values from the graph were used to
187 correlate with the sensory perception.

188

189 **2.3. Structural characterization**

190 2.3.1 Particle size

191 To determine the size of the small spray and big Ca-alginate beads, static light scattering
192 (Malvern MasterSizer 3000, Malvern Instruments Ltd, Worcestershire, UK) was used. The
193 median diameters (D_{50}) of the beads were measured by dispersing them in the aqueous
194 medium. The particle size of the big beads and spray beads are summarized in Table 2.

195

196 2.3.2 Cryogenic-Scanning electron microscopy

197 In order to directly visualize the interaction of Na-alginate or big Ca-alginate beads with *κ*-
198 carrageenan network, cryo-SEM observation was carried out using Quorum PP-2000 system,

attached to the Quanta 200F FEG microscope (FEI Company, Eindhoven, Netherlands) equipped with liquid nitrogen cooled sample preparation and transfer units. Gel samples were fixed onto the sample holder using cryo-adhesive tape. The samples were flash frozen in liquid nitrogen "slush" (−210 °C) and transferred to the cryo preparation chamber. The samples were fractured using a liquid nitrogen-cooled razor blade before having a short sublimation at −95 °C for 4 minutes. Once fractured with a blade and coated with platinum, a section of the sample was inserted into the observation chamber equipped with a SEM cold stage module held at −125 °C, operated at 3 kV in low vacuum mode and equipped with a backscatter detector.

2.3.3 Transmission electron microscopy

Transmission Electron Microscopy (TEM) images was used to visualise the microstructure of the transparent *κ*-carrageenan gel with or without the incorporation of Ca-alginate beads. 10 µL of sample was fixed with 2.5% (v/v) glutaraldehyde in 0.1M phosphate buffer, followed by washing twice in 0.1M phosphate buffer and post fixed in 0.1% (w/v) OsO_4 for overnight. The samples were then carefully exposed to serial dehydration in ethanol (20-100%) before being embedded in propylene oxide-araldite for several hours. Ultra-thin sections (silver-gold 80-100 nm) were deposited on 3.05 mm grids and stained with 8% (v/v) uranyl acetate for 5-120 minutes and lead citrate for 5-30 minutes. The sections were cut on an "Ultra-cut" microtome. Images were recorded using a JEM1400 TEM microscope (JEOL, Massachusetts, USA) with a tungsten filament running at 120 kV.

2.4. Sensory analysis

Quantitative Descriptive Analysis® was performed according to the procedure described by (Stone, Sidel, Oliver, Woolsey, & Singleton, 2008).

Selection of terms and panel training.- A panel of eleven assessors (between 20 and 34 years old) was trained to select the descriptors using the checklist method (Lawless & Heymann, 2010). This study has been reviewed and approved by Faculty Ethics committee at University of Leeds [ethics reference (MEEC 14-014)]. Terms were selected and discussed in an open session with the panel leader. First of all, the assessors were given a brief outline of the procedures and a list of attributes and representative samples; then they were asked to choose and write the most appropriate attributes to describe all the sensory properties of the gels or suggest new ones. The panel leader collected and wrote all the attributes on a board. The panel discussed the appropriateness of the selected attributes, their definitions and procedures of assessing them. At the end of the session, a consensus on the list of attributes and procedures was reached (Table 3); this procedure was proposed by Stone and Sidel (2004) in order to obtain a complete description of a product's sensory properties. The panellists attended eight 30-minute training sessions. Training involved two stages: in the first stage, different samples were tested by the panellists for better understanding of all the descriptors, different tastings were done until the panel was in consensus in its assessments (standard deviation<2). In the second stage, the panellists used a 10 cm unstructured scales to score the selected attributes of the gels. All tests were conducted with samples at 25°C.

Formal assessment. A balanced complete block experimental design was carried out in duplicate (two sessions) to evaluate the samples. The intensities of the sensory attributes were scored on a 10 cm unstructured line scale. Nine samples were evaluated per session. In each session, the samples were randomly selected from each batch, and served in a random order, each on a separate plastic cup identified with random three-digit codes.

2.5. Participant's examination

2.5.1. Recruitment

Eleven participants (between the ages of 18-25, 5 males and 6 females) participated in this study and gave written informed consent before the start of the study. The present part design was approved by Faculty Ethics committee at University of Leeds [ethics reference (MEEC 14-006)].

2.5.2. Eating difficulty ranking

The difficulty perceived was scored by these 11 young participants who did not participate as a trained panel. The model gels were given in a random order inside a plastic cup and participants were asked to order the gels in a scale of 10 cm from easy to difficult, ethics reference (MEEC 14-014)].

2.5.3. Measurment of physical and oral strengths

Physical strength measurements for hand gripping force, tongue pressure and biting force were measured using the methodology described in a previous studies (Laguna, Sarkar, Artigas, & Chen, 2015a, b), all techniques were non-invasive. All these measurements were conducted with an aim to use a homogeneous group of young population having similar levels of capabilities. Briefly, hand gripping force was measured with an adjustable handheld dynamometer (JAMAR dynamometer, Patterson Medical Ltd., Nottinghamshire, UK). To measure the biting forces, a thin flexible force transducer (Tekscan, South Boston, Massachusetts, USA) was used with two adhesive silicon disc (diameter: 1.5 cm, thickness: 0.3 cm to sandwich the force sensor) connected to a multimeter placed between incisors. Finally, for the tongue pressure, the Iowa Oral Performance Instrument (IOPI®, Medical LLC, Redmond, Washington, USA) was used. Previous to using the equipment, each

measurement was demonstrated to the participant by a trained demonstrator and any questions were answered before the conducting the experiments on subjects. The use of the above equipment have been included in the ethics applications [(MEEC 14-014), (MEEC 14-006) and (MEEC 14-018)].

2.5.4. Video recording analysis: Observational study of oral processing and swallowing

Prior to the video recording session, participants had the complete explanation that they will eat different gels in the order they prefer. Participants were aware that the main focus of this video-recording session was to record their mastication and swallowing behaviour. The instructions given to the participants were: "interviewer will ask you to eat and masticate normally food gels while you will be recorded. The time needed to process the food at mouth and the swallowing time will also be recorded".

Videos recorded using camera (Canon Powershot SX500 IS) were analysed frame-by-frame to study the number of chew cycles and swallowing time. One chew cycle refers to the point from the jaw closing after placing the gel inside the mouth up to the upward and the downward mandible movement was completed. To record the time at swallowing, researchers observed two factors: lip seal force increment and consequently down of the lip corners followed by stop of breathing and pharynx movement. The swallowing process was considered finished once the participant had returned to normal breathing, shown by slight opening of the mouth. Oral residence time was defined as the time from the ingestion till the completion of swallowing (Chen & Lolivret, 2011). It is worth pointing out that video recording gives a good estimation of the oral residence time, as compared to invasive techniques such as nose endoscope that allows better visualization of the gastroesophageal junction (Belafsky, Postma, Daniel, & Koufman, 2001; Postma, Bach, Belafsky, & Koufman,

2002; Yamashita, Sugita, & Matsuo, 2013). However the latter does require trained clinicians and local anaesthesia, which was out of scope of the current ethics application.

All tests were conducted with samples at 25°C.

2.6. Statistical analysis

The mean values and standard deviations (SD) were calculated using Microsoft Office Excel 2010. For each trained panel attribute descriptor, two-way ANOVA was applied to check panel performance considering assessors, samples and their interaction as factors. Analysis of variance (one-way ANOVA) was applied to the trained panel in order to study the effect of formulation; least significant differences were calculated by Tukey's test ($p<0.05$). ANOVA tests were done using SPSS (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp). Principal component Analysis (PCA) was done to study the relationships among sample fracture behaviour (large deformation), trained panel characteristics, oral residence time and difficulty perceived. These analyses were performed using XLSTAT 2009.4.03 statistical software (Microsoft, Mountain View, CA).

3. Results and discussion

3.1. Oscillatory deformation

Dynamic strain sweep tests were conducted for all samples at strain amplitudes ranging from 0.01 to 100% (Figure 1) at 1Hz. The linear viscoelastic region (LVR) was defined, within which moduli remained independent of amplitude of oscillation. In all samples with or without the addition of Na or Ca-alginate, this linear region appeared at strains below 0.1%. In the narrow LVR region (extending to only about 1%), native *κ*-carrageenan gels (1-4 wt%) were highly structured and were behaving more solid-like with G' superior to G" (Figures 1A and B). Beyond the critical strain, the elastic structure was broken down, and system behaved

fluid-like when G" exceeded G'. The effect of decreasing *κ*-carrageenan concentration from 4 to 1 wt% increased the deformability of gels such that they could withstand larger strains before the flow occurred, which is in line with the previous finding (Garrec, Guthrie, & Norton, 2013). In Figures 1A and B, LVR was extended for the mixed gels containing *κ*-carrageenan and Na-alginate (M-1*κ*1SAl, M-2*κ*2SAl) towards higher strain values (>10%) than gels containing only *κ*-carrageenan indicating a bicontinuous network (Ould Eleya & Turgeon, 2000). In the systems containing Ca-alginate beads, the critical strain was low (< 1%) indicating a closer packing of the beads within the *κ*-carrageenan gels. Based on similar pattern of strain curves, the samples can be graded into two groups, S-1*κ*1CAl, B-1*κ*1CAl, 2*κ* showing higher G' values and narrow linear spectra versus M-1*κ*1Sal showing the opposite trend (Figure 1A). Similar trend was observed in the strain sweep for higher concentration (Figure 1B); with the S-2*κ*2CAl, B-2*κ*2CAl, 4*κ* showing similar behaviour. The viscoelastic behaviour of these systems appeared to be dominated by the small-deformation properties of the *κ*-carrageenan network. Eventually all the gel networks with the presence of Na-alginate or Ca-alginate beads were fractured at strain levels within 1-20% yielding a dramatic drop in the values of G'.

Using the mechanical spectra, the rheological behaviour of different gels at frequency of 1 Hz is shown in Figure 2. In all gels, except M-1*κ*1SAl, G' exhibited a predominance over G", showing signature of strong gel-like rheological behaviour (Núñez-Santiago, Tecante, Garnier, & Doublier, 2011). The G' increased as a function of concentration following power law of 2.04 (correlation coefficient 0.97) as expected in case of native *κ*-carrageenan gels (Figure 2). This is expected as the aqueous solution of *κ*-carrageenan is transformed into a gel state at a high concentration due to the formation of a three-dimensional network structure induced by the aggregation of double helices (Liu, Chan, & Li, 2015). Presence of Na-alginate (M-1*κ*1SAl, M-2*κ*2SAl) resulted in weakening of the *κ*-carrageenan gel. However,

presence of beads appeared to contribute to slight reinforcement of the *κ*-carrageenan gel, particularly at 2 wt% biopolymer concentration. At higher total biopolymer concentration (4 wt%), G' declined in gels containing Ca-alginate beads and modulus comparable to that of native *κ*-carrageenan could not be achieved, which highlights limited interaction between the beads and the *κ*-carrageenan network.

3.2. Fracture behaviour using large deformation rheology

The mechanical properties of the gels were characterized by compression and penetration test. Figure 3A shows the samples with 1 wt% (1*κ*) and 2 wt% (2*κ*, M-1*κ*1SAl, B-1*κ*1CAl and S-1*κ*1Cal) biopolymer concentrations and Figure 3b shows samples at 2 wt% (2*κ*) and 4 wt% (4*κ*, M-2*κ*2SAl, B-2*κ*2CAl and S-2*κ*2CAl) biopolymer concentrations. All samples appeared to follow a similar trend, with the second peak lower than the first. However statistically (data not shown), sample springiness can be segregated in two groups; the lowest recovery (56-63 %) was observed for samples with beads (small and big: B-1*κ*1CAl, S-1*κ*1CAl, B-2*κ*2CAl and S-2*κ*2CAl) in comparison with the springiness of those samples without beads (88-94%) (1*κ*, 2*κ*, 4*κ*, M-1*κ*1SAl, M-2*κ*2SAl).The peak shape was different, being sharper for the gels containing continuous *κ*-carrageenan network (1*κ*, 2*κ* and 4*κ*) as compared to samples containing beads (in particular B-2*κ*2CAl). As expected the peak force was dependent on *κ*-carrageenan concentration, samples with lower concentration (1-2 wt%) were softer than samples with higher concentration (4 wt%). Mixed gels of Na-alginate and *κ*-carrageenan at 2 wt% biopolymer concentration had peak force comparable to *κ*-carrageenan gels (1 wt%).

Fracture can be defined as the macroscopic breakdown of the matrix (Berg, Sarvimäki, & Hedelin, 2006). In the Figure 4, fracture can be observed as the catastrophic fall after the

maximum penetration force is attained. Penetration test showed similar trend in the force at break (Figure 4), with the hardest sample being 4 wt% *κ*-carrageenan gel (4*κ*), followed by 2 wt% *κ*-carrageenan gel (2*κ*). Presence of big beads (B-2*κ*2CAl) and small beads (S-2*κ*2CAl) caused a decrease in the fracture force. This demonstrates that the beads were not connected to the *κ*-carrageenan gel, and thus weakened the gel network making it less resistive to deformation (Ching, Bansal, & Bhandari, 2016). Detailed information on the compression and penetration test parameters is provided as supplementary information (Tables S1 and S2). Additionally in Figure 5, the fracture point as a function of the matrix inhomogeneity of the gels is shown. The four different categories of samples were classified as a function of their beads contents and the size of the beads. As expected, hardness increased in the native *κ*-carrageenan gels with the biopolymer concentration increment. However, presence of Na-alginate weakened the gel, which is in agreement with small deformation rheology results. This might be attributed to Na-alginate possibly interfering with the incipient coil-to-helix transition during the formation of the *κ*-carrageenan network leading to a weaker gel. From Figure 5, it can be also clearly observed that the presence of Ca-alginate beads resulted in weakening of the structure. It is also worth noting that 1*κ*, M-2*κ*2SAl, S-2*κ*2CAl and B-2*κ*2CAl gels have different levels of inhomogeneity but similar deformation forces, which might be attributed to the mechanical response of the degree of structure. In other words, there was limited interaction between the Ca-alginate beads and the matrix irrespective of the particle size. The beads were unbound to the *κ*-carrageenan gel matrix and thus induced a decrease of the gel modulus (van Vliet, 1988).

3.3. Microstructure

To unravel the structural aspects of the gels, scanning electron micrographs for the native *κ*-carrageenan gels and mixed Na-alginate-*κ*-carrageenan gels (2*κ*, M-1*κ*1SAl, 4*κ*, M-2*κ*2SAl)

of 2-4 wt% total biopolymer concentration gels have been investigated in two magnifications (Figure 6). As shown in Figures 6A1 and A2, the micrographs of native *κ*-carrageenan gels show clear strands forming a network of open large pores around 1–4 μm resembling a honey-comb structure, the pore size decreased and the network became denser with increasing *κ*-carrageenan concentration from 2 to 4 wt%, with formation of fibrillar network structures by a side-by-side association (Figure 6B1 and B2) (Liu, et al., 2015). As shown in higher magnification images, the network strands of native *κ*-carrageenan gels appeared to have a stiff and rigid appearance. The structure of native *κ*-carrageenan gels are in agreement with a previous study (Liu, et al., 2015; Medina-Torres, Brito-De La Fuente, Gómez-Aldapa, Aragon-Piña, & Toro-Vazquez, 2006). Also, the concentration dependence of *κ*-carrageenan network density is in line with the higher G' value and failure strain (Figures 1 and 2), i.e. gels with 4 wt% *κ*-carrageenan were stronger and more deformable as compared with those formed with 2 wt%. Thrimawithana, Young, Dunstan, and Alany (2010) have also reported high tensile properties of such high concentration systems. On the other hand, the mixed Na-alginate-*κ*-carrageenan gels presented an altered structural organization (Figures 6C1, C2, D1 and D2) as compared to *κ*-carrageenan network; latter however remained as the dominant continuous phase. Particularly, looking at the higher magnification images (Figures 6C2 and D2); presence of 1 or 2 wt% of Na alginate appeared to disrupt the continuity of the *κ*-carrageenan network strands and the matrix showed increased degree of broken and/or interrupted junctions or so called "interpenetrating networks" as expected from the decreased modulus of the mixed systems (Ould Eleya, et al., 2000).

Analysing the TEM images, mixed *κ*-carrageenan gels formed a cross-linked network (Figure 7A) as evidenced in cryo-SEM images previously, whilst when calcium alginate beads were included; surface irregularities in the matrix morphology was observed. Particularly, when

422 comparing the gels containing big beads versus small beads (Figures 7B and C), the network
423 irregularities appeared to increase with decrease in particle size. These observations suggest
424 that alginate beads as a function of their increasing surface area were possibly competing for
425 water sorption and interfering with the development of the *κ*-carrageenan network leading to
426 a reduction in the overall mechanical response in terms of force at break (Figure 5). As
427 highlighted in Table 2, the increasing concentration of polymers had an effect on increasing
428 the size of the beads with median diameter of small beads being three times at 4 wt% than
429 that at 2 wt% biopolymer concentration. Even in case of big beads, diameter (D_{50}) was twice
430 at 4 wt% as compared to 2wt% biopolymer concentration. No reduction of the bead size was
431 observed when incorporated in the *κ*-carrageenan continuous phase; however cracks in the
432 surfaces of the beads were evident (Figure 8A and B). Overall, microscopy images support
433 the rheology results of *κ*-carrageenan network whose rigidity was directly affected in
434 presence of Na-alginate. Introducing beads altered the surface regularity of *κ*-Carrageenan
435 (Figure 7) by introducing defects due to the presence of "inactive filler particles" and resulted
436 in a less defined network (van Vliet, 1988). Based on rheology and microstructural results, it
437 can be concluded that chosen gel types covered a wide range of breakdown behaviours,
438 which allowed a broad comparison among gel matrices and yielded conclusive results of the
439 effects of inhomogeneity on both the sensory properties and oral residence time.
440

441 **3.4 Sensory analysis and oral processing**

442 Sensory characterization of the gels was done with the aim to understand if these instrumental
443 mechanical and structural properties can trigger a sensory response. In this section, firstly the
444 sensory perception of the gels (by a trained panel performing QDA™) was analyzed and then
445 oral processing properties of the gels including the time at swallowing on the basis of their
446 structure and rheology were evaluated. It is worth to note that we have focussed on the initial

447 food structure of the gels and the initial degree of inhomogeneity, which might not remain
448 same during the entire oral processing regime. Our goal was to understand the behaviour of
449 gels with different initial structure (with different degree of homogeneity) when oral
450 deformation and fracture occurred.
451
452 3.4.1 Quantitative descriptive analysis (QDA)
453 The mean scores of the sensory analysis results are plotted in Figure 9. As an obvious
454 consequence, gels with no beads added (*κ*, M-*κ*SAl) had no particle presence visually, or in
455 mouth. Regarding the particle size, panellists scored no significant difference between small
456 and big beads (B-*κ*CAl or S-*κ*CAl). Native *κ*-carrageenan gels (1*κ*, 2*κ*, 4*κ*) were scored as
457 transparent. Mixed gels (M-*κ*SAl) or gels with small beads (S-*κ*CAl) were considered as
458 opaque. Manual hardness (making pressure with the spoon) and oral hardness (making
459 pressure with the tongue) had similar values. Hardness perception can be graded in three
460 different groups, the softer ones were samples M-1*κ*1SAl<S-1*κ*1Al<1*κ*<B-1*κ*1CAl, followed
461 by M-2*κ*2SAl<S-2*κ*2CAl<B-2*κ*2CAl and the hardest being 2*κ*<4*κ*.
462 The initial matrix homogeneity was judged by panellists according to the presence or absence
463 of beads. Regarding cohesiveness, the only samples considered (statistically significant) to be
464 non-cohesive were those with big beads. Mixed Na-alginate- *κ*-carrageenan gel samples (M-
465 *κ*SAl) were scored as adhesive or sticky, and were also considered to be higher in mouth
466 coating feeling and after taste. The samples that needed more number of chews were the
467 hardest ones being (2*κ* and 4*κ*). Samples that require the lowest number of chews were M-
468 1*κ*1SAl, 1*κ*, M-2*κ*2SAl and S-1*κ*1Al.
469

3.4.2 Participants characteristics

Participant's characteristics chosen for this study are shown in Table 4. All the participants were young and in good health status. The magnitudes of dominant hand grip forces correspond to the normative grip strength data (Budziareck, Pureza Duarte, & Barbosa-Silva, 2008) and tongue pressure values were in line with results of young population (Alsanei & Chen, 2014). Bite force is known to be dependent on the geometry of the instrument as well as the position where it is located (Ferrario, Sforza, Serrao, Dellavia, & Tartaglia, 2004; Gibbs, Anusavice, Young, Jones, & Esquivel-Upshaw, 2002; Laguna & Chen, 2016; Laguna, et al., 2015a). Higher forces have been reported in young adults in some previous studies (Chen, Pröschel, & Morneburg, 2010; Tortopidis, Lyons, Baxendale, & Gilmour, 1998) whilst our results are within the range of values obtained by Fernandes, Glantz, Svensson, and Bergmark (2003) using a similar flexisensor placed in the incisors.

3.4.3 Oral residence time

During the food oral processing, tactile and kinaesthetic receptors continuously inform the central nervous system adjusting the masticatory actions to the changes in the food physical properties (Trulsson, 2006; Türker, Sowman, Tuncer, Tucker, & Brinkworth, 2007). This sensory feedback also determine the duration of chewing and the number of cycles until swallowing (Hiiemae, et al., 1996). In accordance with previous study (Engelen, Fontijn-Tekamp, & Bilt, 2005), in Figure 10A, it can be observed how the time in mouth is correlated (0.709) with time at swallow, so those samples that needed longer time at mouth were continuously being chewed. (Peyron, Lassauzay, & Woda, 2002) also stated that not only for harder products, there occurs an increment of number of chews, there is also an increase of the muscle activity during every stroke. They also reported a linear correlation between muscle activity and food mechanical properties. We believe that this extra effort needed to

masticate harder samples could be linked with the difficulty perception. Çakır, et al. (2012) affirmed that there is a link between the duration of mastication with the easiness at which food is broken down and transformed into a cohesive bolus. In the same graph (Figure 10B), the difficulty perceived is plotted against the number of chews, and it can be observed that there is a relation, but is lower than the correlation between time and number of chews. It is worth noting that there might be other phenomenon that might be influencing the difficulty perception.

In Figure 10B, the sensory hardness (correlated with the instrumental maximum force at break r=0.880 according to Persons' correlation) at different levels of matrix inhomogeneity was plotted against the oral residence time. Here, matrix inhomogeneity is defined as the presence of perceivable semi solid gel particles within another gel matrix. In other words, the least inhomogeneous is the gel being prepared with one biopolymer, and the most inhomogeneous is the gel being prepared with big Ca alginate beads. It can be observed that the increment of κ-carrageenan concentration resulted in an increase in cross-linked network density, and the time in mouth increased significantly with the hardness perceived. The inhomogeneity effect has been studied using two independent factors: sensory hardness (score by panellist and defined by the panel as "force required to break the gel with the tongue") and time at swallow (time needed by participants to swallow). It was interesting to note that even with same level of hardness, the time in mouth increased with the increasing degree of matrix inhomogeneity. Gels such as 1κ, M-1κ1SAl, S-1κ1CAl, B-1κ1CAl had a sensory hardness lower than 20 points whilst the oral residence times varied from 4 seconds (1κ) to 10 seconds (B-1κ1CAl). With higher concentration of biopolymer, same influence of the degree of inhomogeneity was observed, for example 4κ was the hardest sample, but the oral residence time was lower than B-2κ2Cal.

3.6. Correlation between food structure, sensory properties and oral processing parameters

In order to summarize all the information captured during chewing and swallowing of the gels by young participants, a principal component analysis (PCA) was plotted with the parameters obtained by the trained panel (Figure 11) and instrumental analysis. It can be seen how gels with initially different degrees of inhomogeneity and biopolymer concentrations spread along the PCA. Also, it can be observed that the results of trained panel (marked in the PCA as TP) on the quantification of attributes to characterize the sample were higher and not necessarily predicted by instrumental parameters (Takahashi, Hayakawa, Kumagai, Akiyama, & Kohyama, 2009).

The PC1 explains 46% of the PCA. Time at swallow, the difficulty perceived and the number of chews appeared in the same PC quadrant, suggesting the positive relations between them. Interestingly, the samples associated with these three factors are the ones containing beads (B-1κ1CAl, B-2κ2CAl and S-2κ2SAl), so presence of calcium alginate beads as opposed to Na-alginate in gels increased the time in mouth, number of chews and difficulty perception. Opposite attributes to difficulty perception was the mouth coating and adhesiveness effect. For the gels tested, samples that were more adhesive and had a mouth-coating feeling were considered to be easier to swallow. In other words, gel samples, which were excessively crumbly were difficult to manipulate in mouth to form a safe bolus to be swallowed. The harder and homogeneous native κ-carrageenan gels (2κ and 4κ) were considered the most "chewy" samples by panellists. The mixed gels containing both biopolymers were the softest and easy to eat (M-2κ2SAl and M-1κ1SAl) probably because they were easy to form a cohesive bolus, and they seemed to provide mouth coating and adhesiveness to the oral mucosa.

The second component of the PCA explains the 36.25% of the sample behaviour and clearly separates the samples in the area of continuity of the gel network. In the positive axis, the gels with different size of beads and in the negative axis the more homogeneous gels with *κ*-carrageenan or the mixed Na-alginate-*κ*-carrageenan gels appeared. It was evident that time at swallow, number of chews and difficulties perceived were more related with degree of matrix inhomogeneity than with hardness (as indicated by trained panel or instrumental analysis). In summary, this result validates the initial hypothesis and clearly suggests that the degree of structure can play an important role in the fracture of the gels affecting the oral processing behaviour and oral residence time.

4. Conclusions

Bolus swallowing is a complex process that has been studied mainly from human physiology and coordination point of view by clinicians. It is well known that food consistency affects the risk of aspiration, and increasing the time at mouth has been largely addressed with viscosity optimization. However, use of thickeners alone can result in a monotonous diet. More importantly, beside rheology, the degree of structure is also an essential variable in oral processing. In the present study we propose a new approach to increase the oral residence time by designing model mixed biopolymer gels with initially different degrees of inhomogeneity. Based on the results highlighted, similar sensory effect on delaying the food entrance into the pharynx and increasing the oral residence time can be achieved by suitable matrix design with incorporation of model alginate beads. This study has shown that not only the consistency increment can help in the designing food for population with swallowing disorders; the matrix heterogeneity does influence the chewing and oral residence time. Future work is directed to investigate the impact of such gels with different degrees of inhomogeneity in oral processing of elderly population who are physically weaker than the

young participants. This novel insight of incorporating structuring defects in gel can be an effective design strategy for future food formulation for elderly.

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753 **Table 1**

754

Sample name	Concentration (wt%)		Presence of calcium alginate beads in the gels
	κ-Carrageenan	Sodium alginate	
1*κ*	1.0	-	none
2*κ*	2.0	-	none
4*κ*	4.0	-	none
M-1*κ*1SAl	1.0	1.0	none
M-2*κ*2SAl	2.0	2.0	none
B-1*κ*1CAl	1.0	1.0	Extruded calcium alginate beads (syringe)
B-2*κ*2CAl	2.0	2.0	Extruded calcium alginate beads (syringe)
S-1*κ*1CAl	1.0	1.0	Spray calcium alginate beads
S-2*κ*2CAl	2.0	2.0	Spray calcium alginate beads

755

Table 2.

Bead size	B-1*κ*1CAl	B-2*κ*2CAl	S-1*κ*1CAl	S-2*κ*2CAl
D_{50} (μm)	1210	2380	56.9	185

758 **Table 3**

Attributes	Definition	Technique	
Using the spoon			
Opacity	Degree to which light is not allowed to travel through	Observation of the gel inside a glass and evaluation of their opacity Scale: from "transparent" to "opaque"	
Particle presence	Visualization of particles number in the gel matrix	Observation of the gel inside a glass and evaluation of their particles Scale: from "none" to "a lot"	
Particle size	Visualization of particles size in the gel matrix		
Hardness (spoon)	Force required cutting with the spoon the gel.	Use the spoon perpendicularly to cut the gel up to arrive to the bottom of the glass containing the gel. Scale: from "soft" to "hard"	
Placing the gel in the mouth			
Elasticity	The degree to which the sample returns to its original shape	Place the sample between the tongue and the palate, and partially compress against the palate	
Hardness at mouth	Force required to break the gel with the tongue	Place the sample between the tongue and the palate, and compress firmly against the palate till the gel breaks	
Brittleness	Fracture after small compression	Evaluate how quick the product breaks when crushed	Scale: from "not" to "very"
Inhomogeneity	Number of non-continuous phase (particles) felt at mouth	Feeling of the gel rubbing with the tongue against the oral mucosa	
Cohesiveness	The amount of chewed sample that holds together		
Adhesiveness	Degree to which samples stick to your tongue, palate and teeth		

Chewiness	The number of chews necessary to chew a sample till it is ready for swallowing	Chew the sample . Scale: from “low” to “high” number	
Mouth coating	Sensation of a layer covering the oral mucosa	Film sensation inside the mouth Scale: from low number to high number	
Particle presence	Feeling the number of particles in the gel	Feeling of the gel paticles rubbing with the tongue against oral mucosa	Scale: from “not” to “a lot”
Particle size	Feeling of the size of particles in the gel		Scale: from “small” to “big”
After feeling			
Mouth coating	Sensation of a layer covering the oral mucosa after swallowing/spitting the gel	Film sensation inside the mouth after swallow/spit Scale: from “not” to “very”	
Mouth watering	Watery sensation or fresh palate sensation after swallowing/spitting the gel	Evaluation of the degree of watery feeling in the mouth Scale: from “not” to “very”	

759

760 **Table 4**

761

	N	Age (years)	Right hand force (kg)	Left hand force (kg)	Tongue pressure (kPa)	Bite force (kg)
Male	5	23.2 (2.05)	46.61 (7.2)	45.59 (8.7)	50.00 (5. 7)	5.92 (5.0)
Female	5	22.6 (1.67)	22.63 (5.5)	21.93 (4.7)	40.07 (14.7)	3.21 (1.3)

762 Values in parenthesis are standard deviations.

763

Table S1

Sample name	Gradient (N/s)	Force (N)	Area (N.mm)
1κ	15.28^{a} (3.70)	45.26a^{b} (12.64)	71.42^{a} (26.29)
2κ	9.64^{a} (3.62)	19.53^{a} (8.70)	41.79a (15.03)
4κ	20.62^{ab} (13.41)	44.95^{ab} (22.78)	132.15 (36.92)
M-1κ1SAl	9.73^{a} (3.65)	39.24^{ab} (17.60)	119.37^{ab} (44.70)
M-2κ2SAl	76.79^{d} (9.99)	267.46^{d} (56.79)	502.42^{cd} (105.93)
B-1κ1CAl	80.01^{d} (23.53)	215.68^{d} (77.11)	599.95^{d} (151.53)
B-2κ2CAl	33.03^{ab} (3.92)	72.04^{b} (5.35)	200.00^{b} (9.91)
S-1κ1CAl	48.67^{c} (18.76)	145.24^{c} (47.14)	441.48^{c} (108.81)
S-2κ2CAl	270.25^{e} (12.28)	882.87^{f} (42.54)	2184.78^{e} (202.56)

Values in parenthesis are standard deviations. Means in the same row with the same letter do not differ significantly ($p > 0.05$) according to Tukey's test.

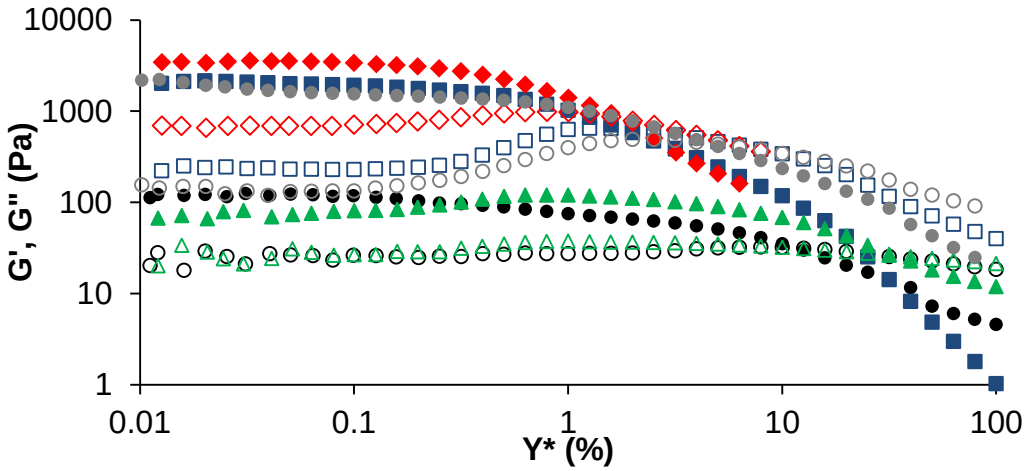
Table S2

Sample name	Force (N)	Area 1 (N.mm)	Area 2 (N.mm)
1κ	5.77^{a} (1.00)	1.30^{a} (0.17)	0.77^{a} (0.11)
2κ	2.46^{a} (0.53)	0.81^{a} (0.16)	0.28^{a} (0.05)
4κ	5.20^{a} (0.99)	2.76^{ab} (1.03)	0.91^{a} (0.27)
M-1κ1SAl	9.32^{a} (2.62)	4.57^{abc} (0.91)	1.27^{a} (0.22)
M-2κ2SAl	33.90^{c} (9.24)	11.74^{cd} (6.50)	4.80^{b} (1.64)
B-1κ1CAl	20.74^{b} (1.59)	6.95^{bcd} (0.44)	4.76^{b} (0.30)
B-2κ2CAl	30.86^{c} (10.54)	11.67^{e} (3.36)	4.80^{b} (1.95)
S-1κ1CAl	18.66^{b} (5.48)	8.87^{e} (1.90)	3.42^{b} (0.77)
S-2κ2CAl	117.05^{d} (5.88)	38.91^{e} (3.54)	27.56^{c} (2.20)

Values in parenthesis are standard deviations. Means in the same row with the same letter do not differ significantly ($p > 0.05$) according to Tukey's test.

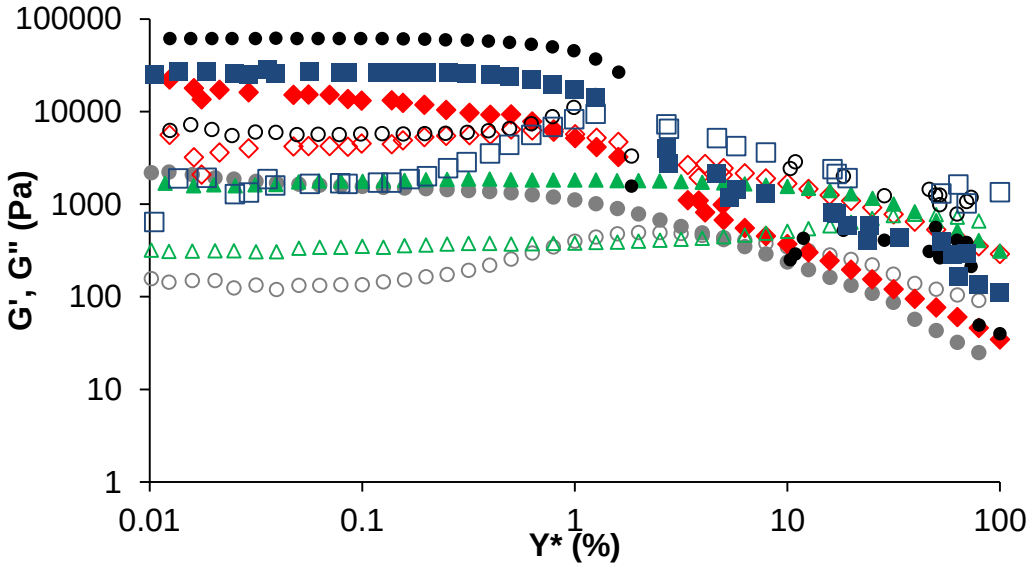
Figure 1.

(A)



G': 1κ (●) B-1κ1CAl (■) M-1κ1SAl (▲) S-1κ1CAl (♦) 2κ (●)
G": 1κ (○) B-1κ1CAl (□) M-1κ1SAl (Δ) S-1κ1CAl (◊) 2κ (○)

(B)



G': 4κ (●) B-2κ2CAl (■) M-2κ2SAl (▲) S-2κ1CAl (♦) 2κ (●)
G": 4κ (○) B-2κ2CAl (□) M-2κ2SAl (Δ) S-2κ1CAl (◊) 2κ (○)

Figure 2.

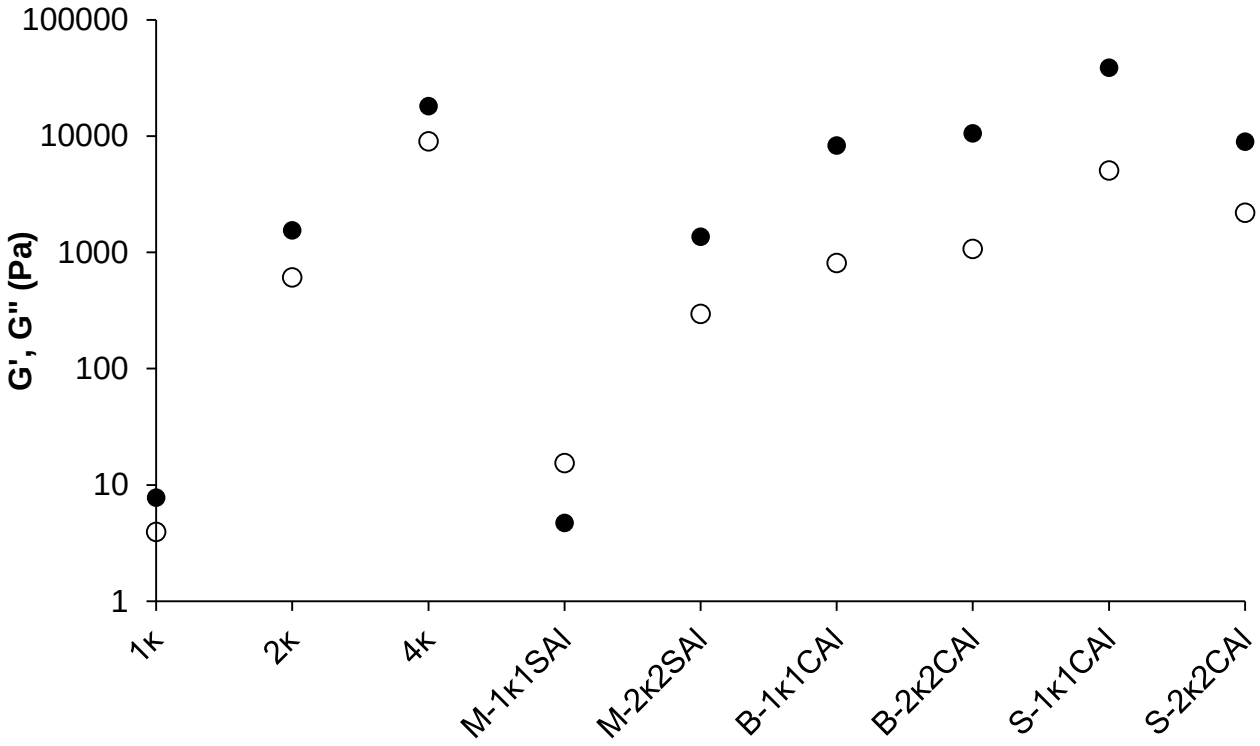

100000
10000
1000
100
10
1
G', G" (Pa)
1k
2k
4k
M-1k1SAl
M-2k2SAl
B-1k1CAl
B-2k2CAl
S-1k1CAl
S-2k2CAl

Figure 3.

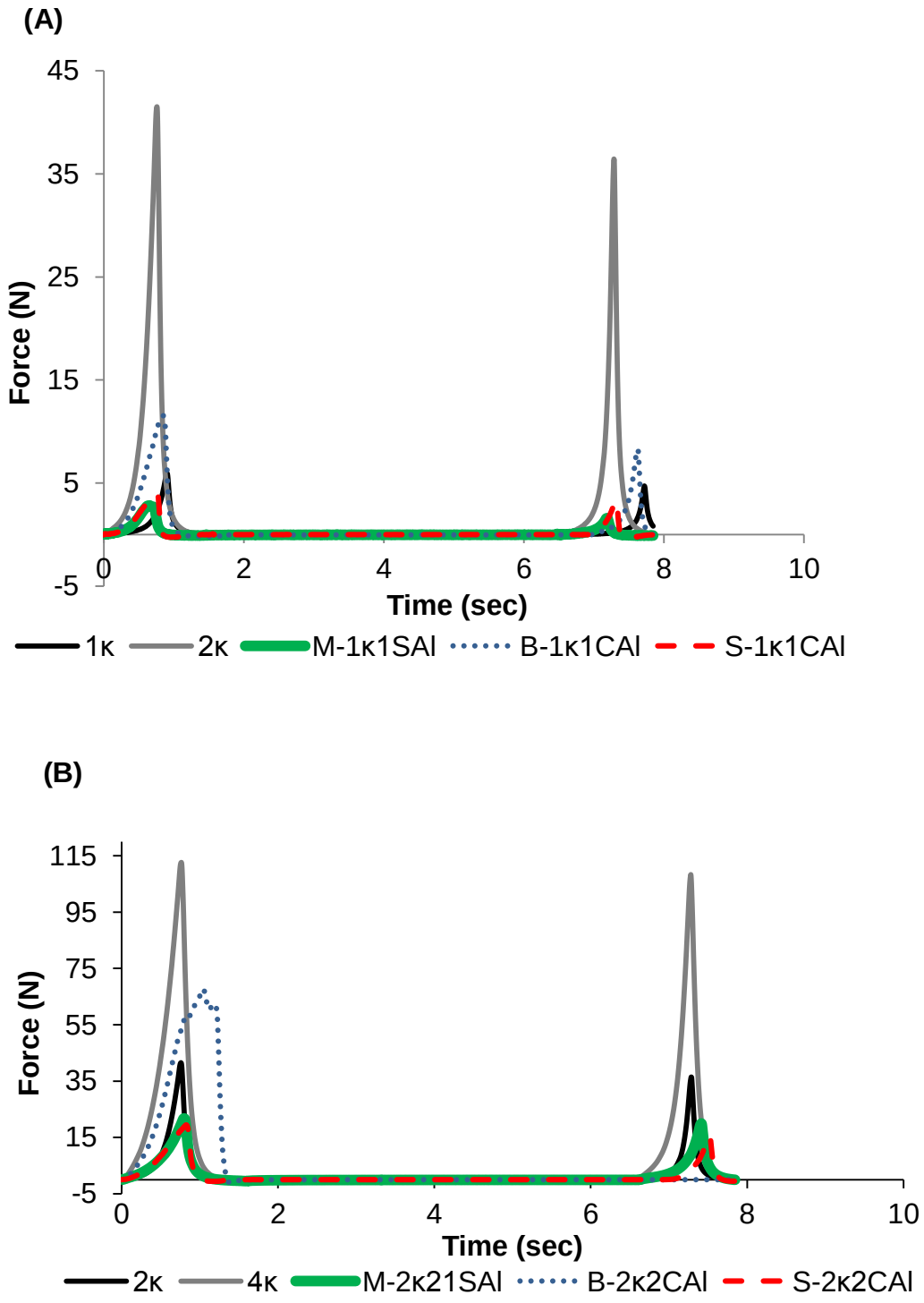
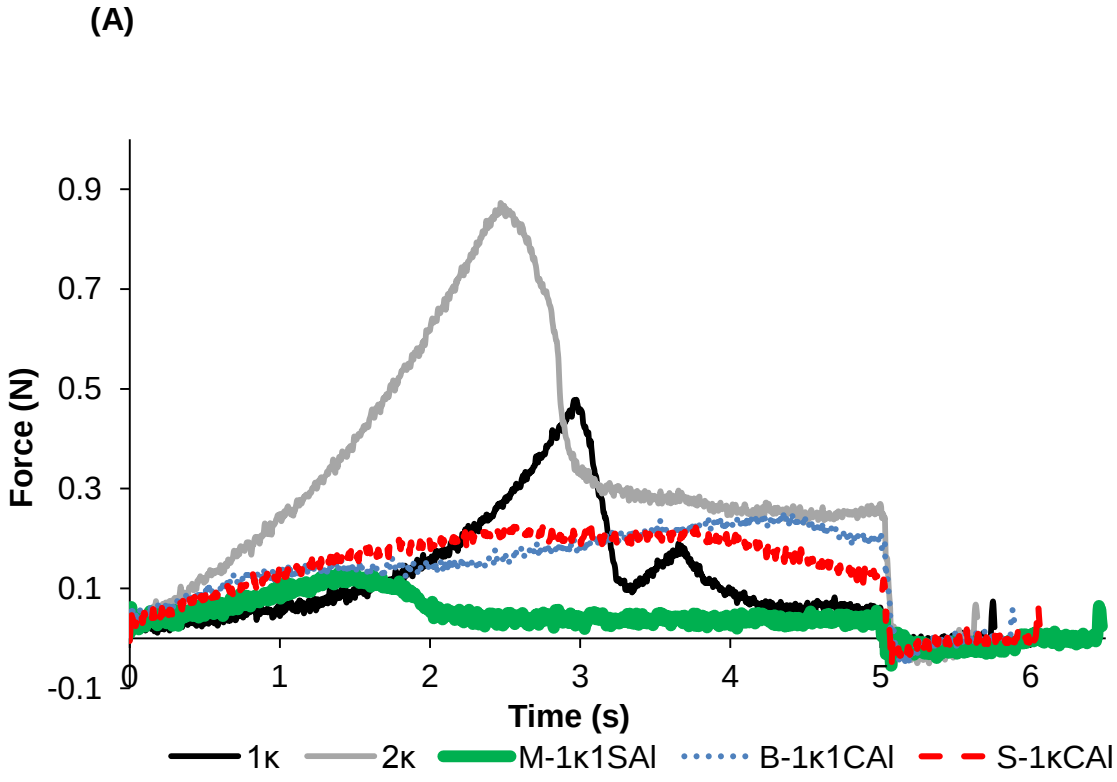
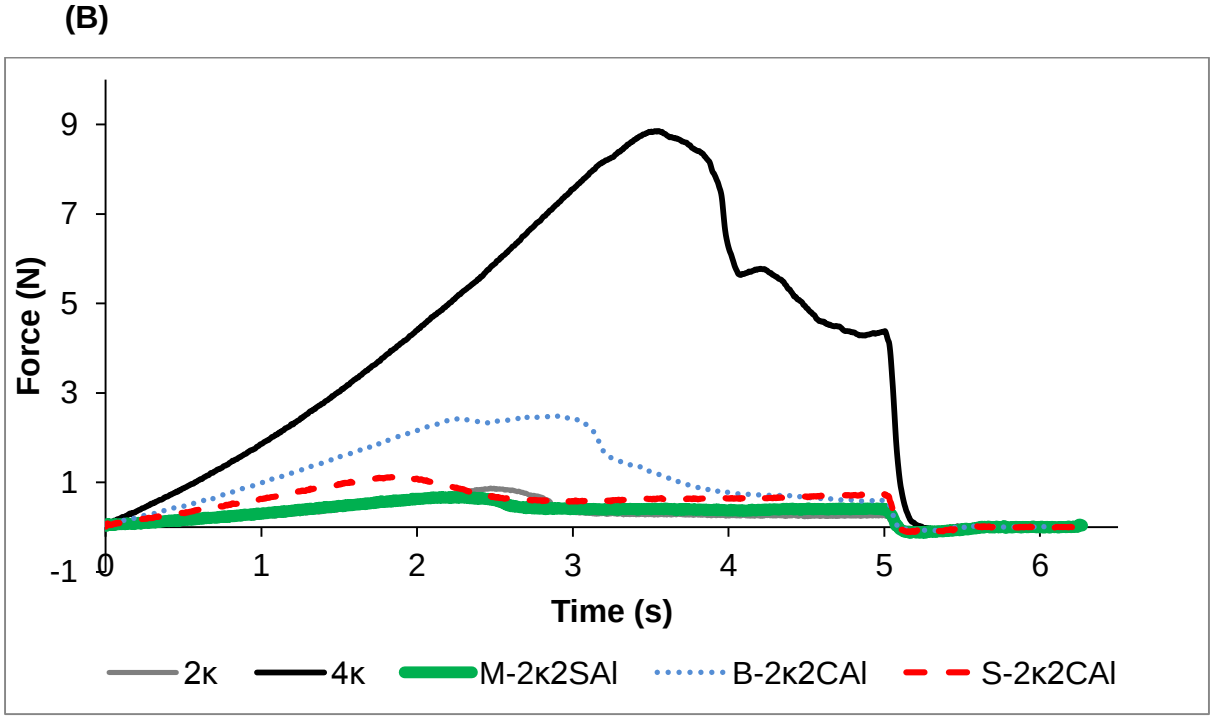
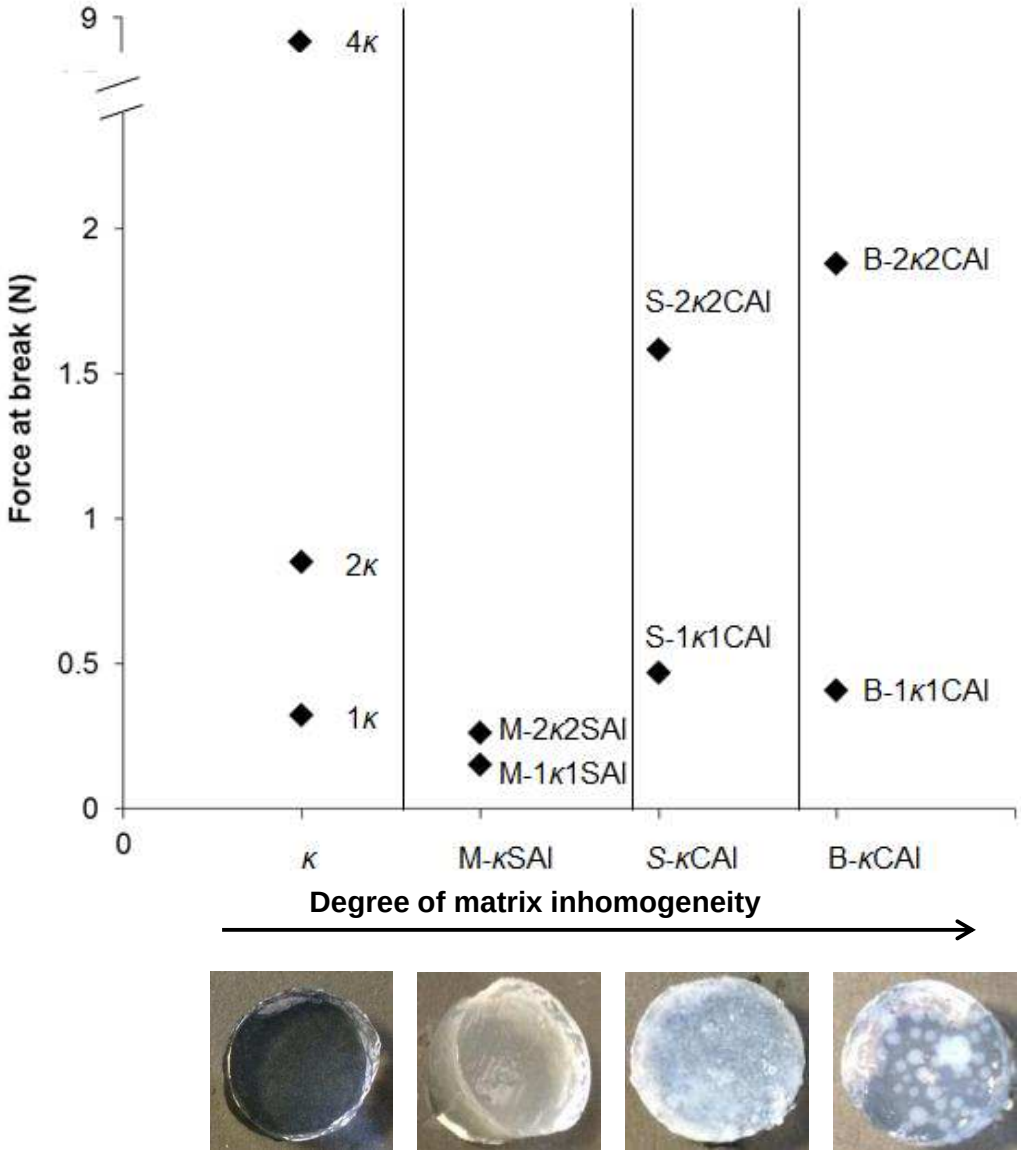

(A)
45
35
25
15
5
-5
Force (N)
0
2
4
6
8
10
Time (sec)
1κ
2κ
M-1κ1SAl
B-1κ1CAl
S-1κ1CAl
(B)
115
95
75
55
35
15
-5
Force (N)
0
2
4
6
8
10
Time (sec)
2κ
4κ
M-2κ21SAl
B-2κ2CAl
S-2κ2CAl

Figure 4.


(A)
0.9
0.7
0.5
0.3
0.1
-0.1
Force (N)
0
1
2
3
4
5
6
Time (s)
1κ
2κ
M-1κ1SAl
B-1κ1CAl
S-1κCAl

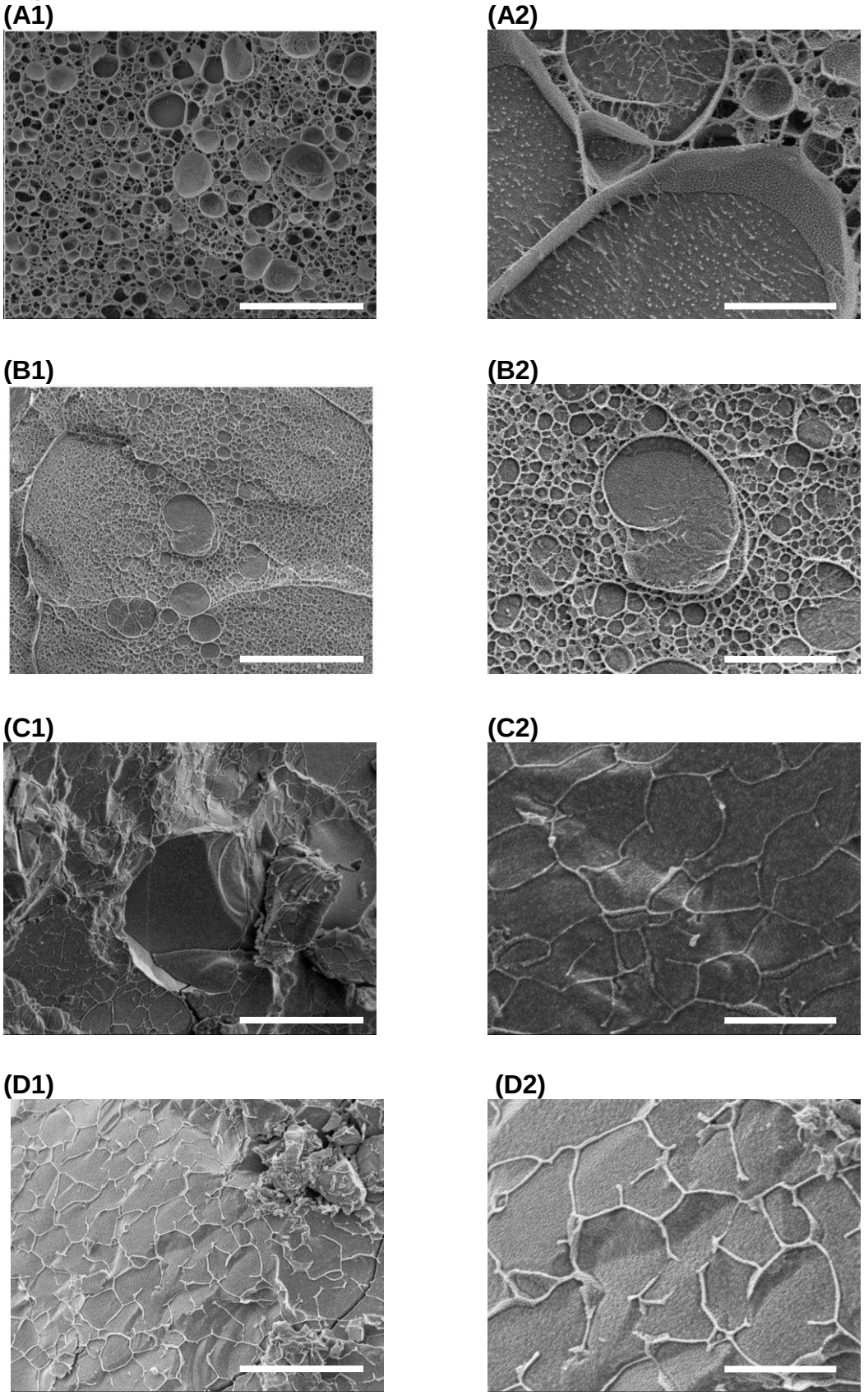

(B)
9
7
5
3
1
-1
Force (N)
0
1
2
3
4
5
6
Time (s)
2κ
4κ
M-2κ2SAl
B-2κ2CAl
S-2κ2CAl

803 **Figure 5.**



804

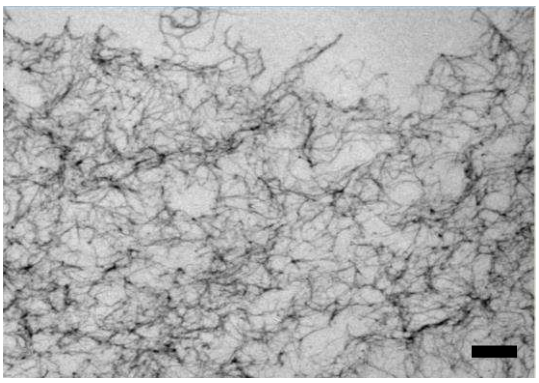
805 **Figure 6.**



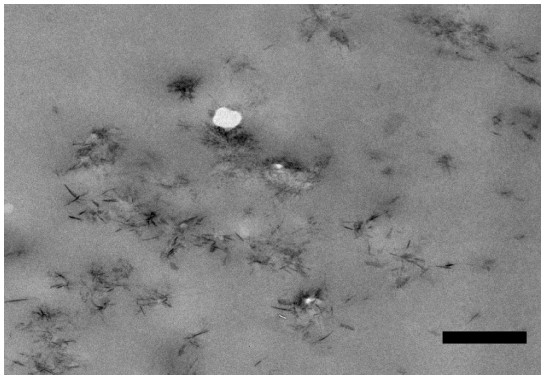
806

807 **Figure 7.**

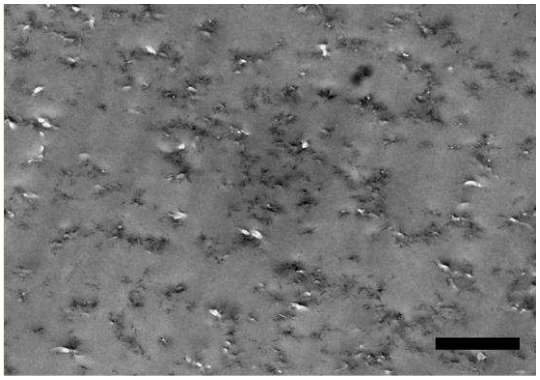
(A)



(B)



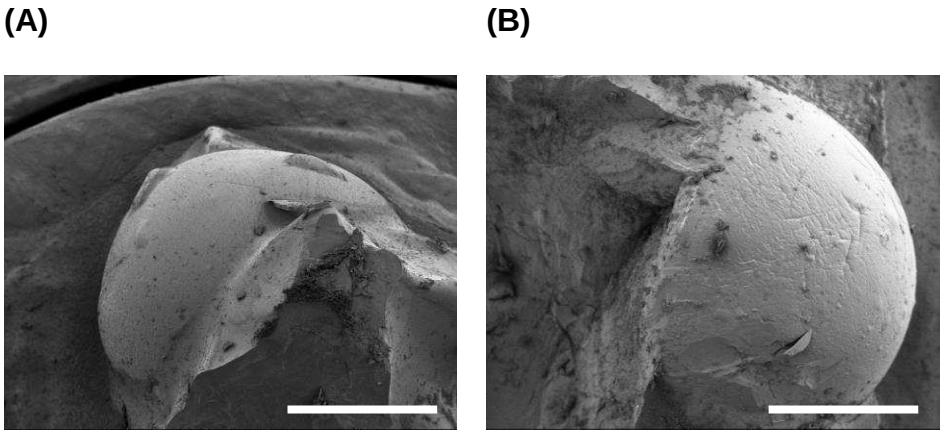
(C)



808

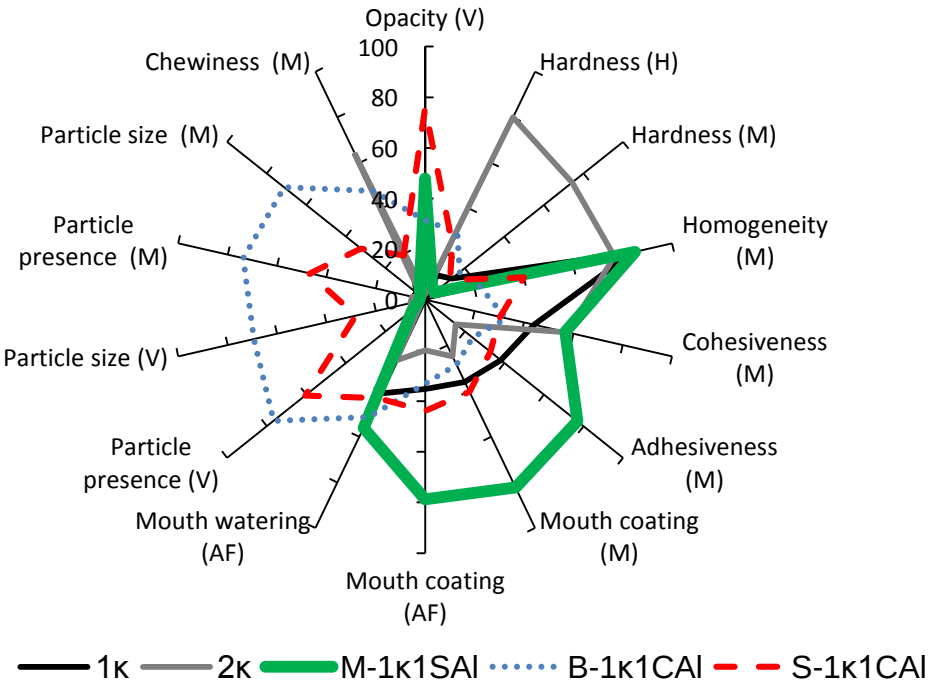
809

Figure 8.



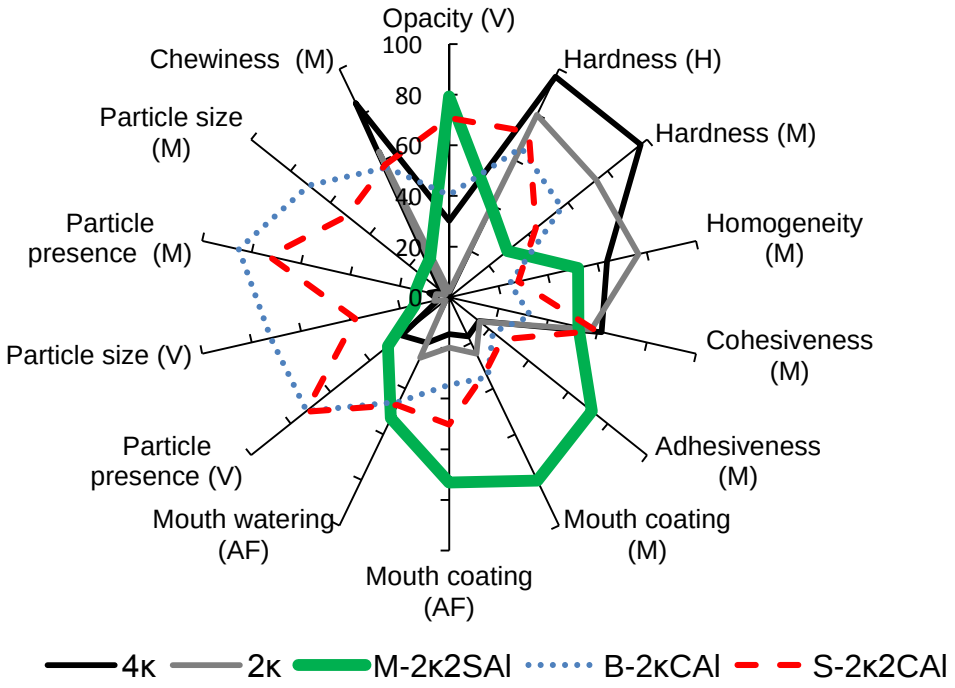
814 **Figure 9.**

(A)



815

(B)

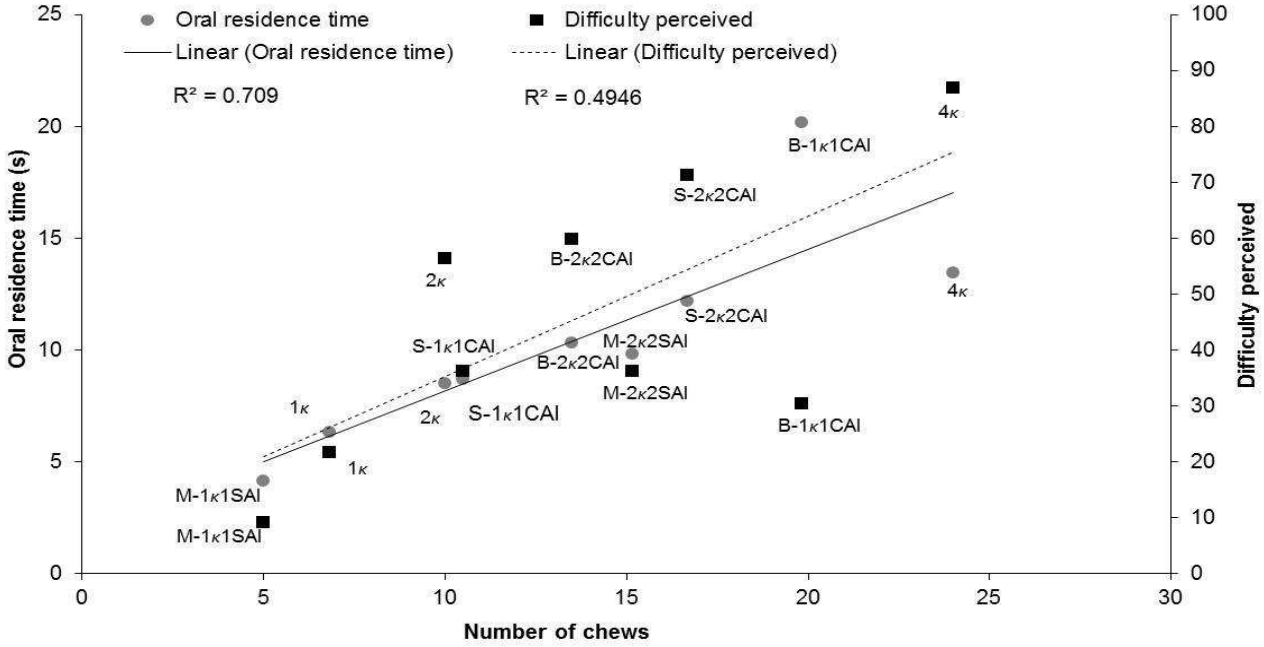


816

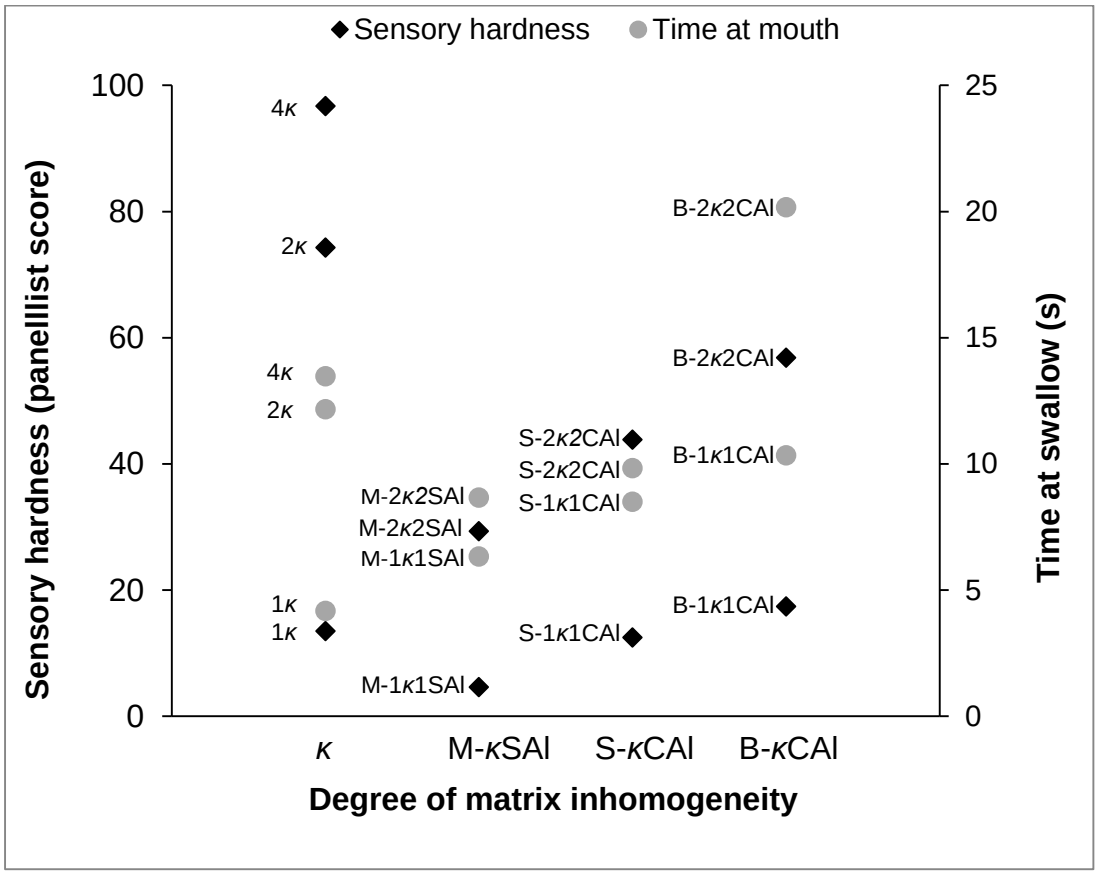
817

Figure 10.

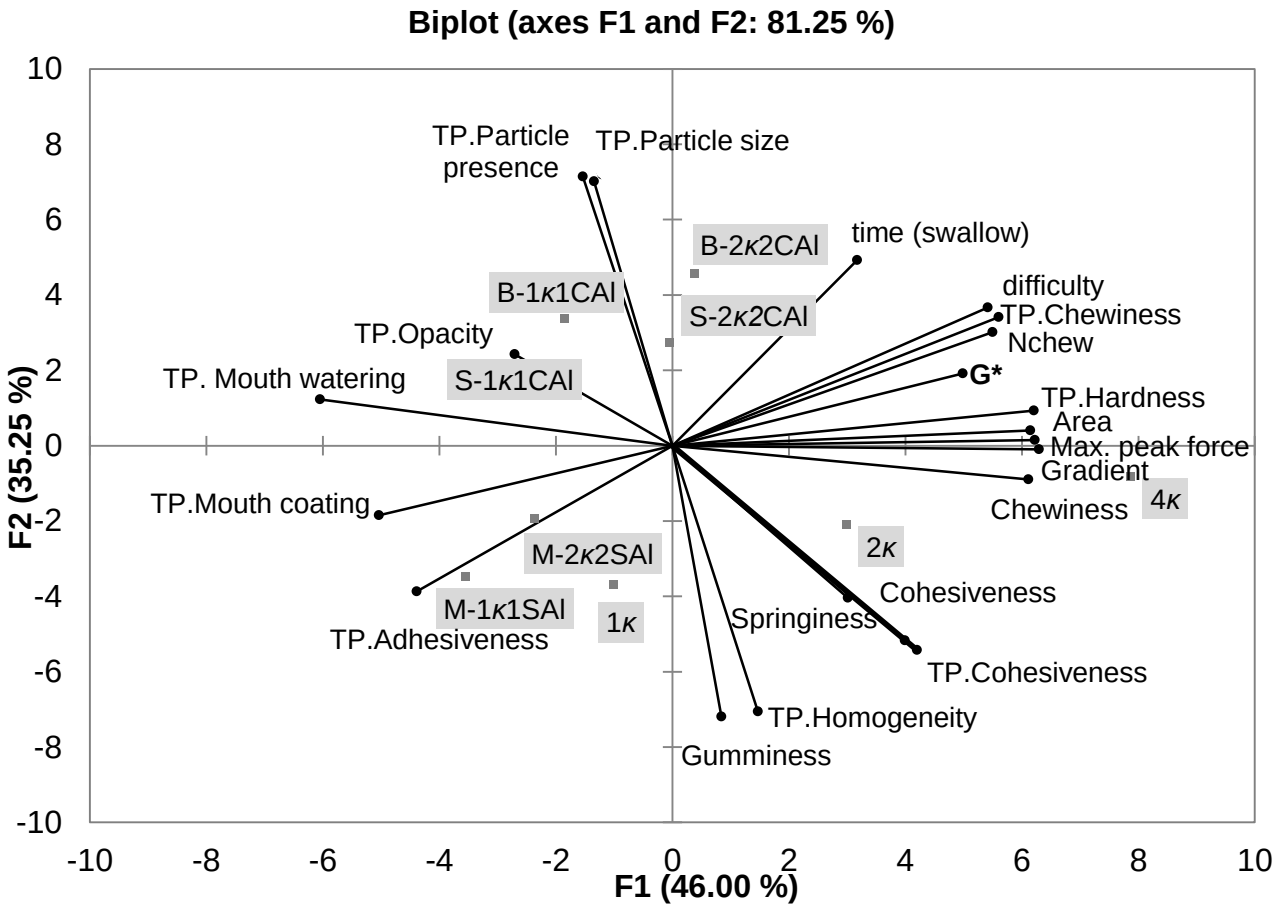
(A)


Oral residence time
Difficulty perceived
Linear (Oral residence time)
Linear (Difficulty perceived)
R² = 0.709
R² = 0.4946
Oral residence time (s)
Difficulty perceived
Number of chews
M-1κ1SAl
M-1κ1SAl
1κ
1κ
2κ
S-1κ1CAl
S-1κ1CAl
M-2κ2SAl
B-2κ2CAl
M-2κ2SAl
2κ
B-2κ2CAl
S-2κ2CAl
4κ
B-1κ1CAl
S-2κ2CAl
4κ
B-1κ1CAl

(B)


Sensory hardness
Time at mouth
Sensory hardness (panellist score)
Time at swallow (s)
Degree of matrix inhomogeneity
κ
M-κSAl
S-κCAl
B-κCAl
4κ
2κ
4κ
2κ
1κ
1κ
M-1κ1SAl
M-2κ2SAl
M-2κ2SAl
M-1κ1SAl
S-2κ2CAl
S-2κ2CAl
S-1κ1CAl
S-1κ1CAl
B-2κ2CAl
B-2κ2CAl
B-1κ1CAl
B-1κ1CAl

824 **Figure 11.**



825

826