Immobilization of gluten in spherical matrices of food-grade hydrogels

Marloes A. Reus[a,*], Georgios A. Krintiras[a], Georgios. D. Stefanidis[a,b], Joop H. ter Horst[c], Antoine E.D.M. van der Heijden[a,d]

[a] Delft University of Technology, Process & Energy Department, Intensified Reaction & Separation Systems, Leeghwaterstraat 39, 2628 CB, Delft, The Netherlands. *M.A.Reus@tudelft.nl.

[b] Katholieke Universiteit Leuven, Chemical Engineering Department, Willem de Croylaan 46, 3001 Leuven, Belgium.

[c] University of Strathclyde, Strathclyde Institute of Pharmacy and Biomedical Sciences, EPSRC Centre for Innovative Manufacturing in Continuous Manufacturing and Crystallisation (CMAC), Technology and Innovation Centre, 99 George Street, Glasgow G1 1RD, U.K.

[d] TNO Technical Sciences, P.O. Box 45, 2280 AA, Rijswijk, The Netherlands.

Abstract

The aim of this paper is to produce spherical encapsulates of wheat gluten in a food-grade biopolymer for preparing sheared meat analogs, in order to prevent instant fibrilization of the gluten during a pre-mixing step. The hydrogel should release the gluten inside the Couette Cell, as a result of the higher temperature and shear in the process. *Both sodium alginate and* κ-carrageenan were used as encapsulants. Spherical particles of hydrogel-gluten mixtures were produced by means of a *dripping method using an encapsulator. While the particle properties of* κ-carrageenan surpassed those of alginate in terms of controlled release of the core, the particle production using the e*ncapsulator was more complicated. With* κ-carrageenan, a layer of oil on top of the cross-linking bath fluid, as well as through the outer orifice of a concentric nozzle were required to obtain a good sphericity of

the particles. For the alginate particles the use of oil was not necessary. Gluten loadings of 7 % w/w were achieved with 1.5 % w/w alginate and with 2 % w/w κ-carrageenan. The water content of the particles can be easily controlled by a subsequent partial drying step. A mixture of Soy Protein Isolate (SPI) and particles was sheared in the Couette Cell. Controlled release of the gluten from the alginate particles was not achieved properly by temperature or shear. The controlled release of the gluten was achieved at the processing condit*ions only with* κ-carrageenan. Some fibrilization was observed in the sheared product, but the macrostructure was not yet well developed. However, an optimization of the shearing process for the use of the particles may lead to an improved structure for the meat analogs.

Practical applications

This paper investigated the effect of encapsulation in hydrogels on the fibrilization behavior of wheat gluten upon contact with water. A cheap and easily scalable dripping technique was used to create spherical particles in which the gluten did not fibrilize, although the coating material consists of ≥95% of water. Upon reaching the process conditions in the shearing device, the gluten are released and able to form fibers. The results show that hydrogels can mechanically protect the core and act as a delivery structure. The protective and carrier functions of the hydrogel can alternatively be used for cores like food additives (e.g. vitamins) or even to pharmaceutical ingredients, not only for the production of meat analogs, but also in other food applications.

Key words: Immobilization, gluten, soy, low shear, fibrilization, hydrogel

1 Introduction

Meat analogs are an increasingly welcome alternative to meat for instance in view of animal welfare (Hughes, 1995) and sustainability (Steinfeld et al., 2006). However, many of the products currently on the market do not reflect the properties of meat to a satisfactory extent (Hoek et al., 2011): Meat analogs lack the juiciness of meat, which follows from its characteristic fibrous structure.

A novel process was developed for the production of highly fibrous meat analogs, using the lab-scaled Couette Cell device (Krintiras et al., 2015). This process achieves meat-like structure formation by applying simple shear flow and heat to plant protein suspensions, resulting in the formation of fibers, which enhance the structure and mouthfeel of the product (Krintiras et al., 2014). During the mixing step of the ingredients prior to loading of the Couette Cell, soy protein isolate (SPI) is premixed with water and left to rest. However, upon addition of the vital wheat gluten (WG), instant fibrilization takes place (Abang Zaidel et al., 2008), forming a sticky gel and local networks. These effects are undesired, since they lead to material losses, as in gluten sticking to the mixing container and spatula. This can be prevented if the gluten could be immobilized and only be released during processing under simple shear and heat.

Microencapsulation is often used to provide such isolation and release functions (Ma, 2014, Wieland-Berghausen et al., 2002, Elzoghby et al., 2011, Zandi, 2016). Hydrogels form a class of materials that is frequently used as encapsulant in biological and pharmaceutical systems (Doherty et al., 2011, Li et al., 2015, Matalanis et al., 2011, Mazzitelli et al., 2008) and would be able to fit the requirements for the gluten encapsulation. The polymers in the hydrogels can hold a large quantity (at least 70%) of water within their three-dimensional structure due to the hydrophilic parts of

76 the molecules (Bai et al., 2015). The open, porous structure does not only allow for
77 the presence of water, but can also provide support to other materials, e.g. cells (Orive
78 et al., 2006, Orive et al., 2003), drugs or peptides (Orive et al., 2006, Zhou et al.,
79 2001).
80 Our aim is to produce spherical encapsulates of gluten in food-grade hydrogel, which
81 release the gluten from the particles at the processing conditions of the meat analog
82 shearing process. The encapsulation step should prevent the gluten from fibrilizing
83 upon contact with water during the premixing step and facilitate easy loading of the
84 formulation into the Couette Cell. The encapsulates should release the gluten inside
85 the Couette Cell, as a result of the higher temperature and shear in the process so
86 structure formation can be achieved. Calcium cross-linked alginate and κ-carrageenan
87 hydrogels are used for the gluten immobilization, because both systems rapidly form
88 rigid gels upon cross-linking or cooling, enabling the product to resist the forces
89 exerted on the particles during mixing and loading. Additional and equally important
90 reasons are that they are accepted in the food industry (Tecante and Santiago, 2012,
91 Keppeler et al., 2009) and that they are expected to release the gluten after application
92 of the high temperature (Mangione et al., 2003) or shear (Papageorgiou et al., 1994)
93 conditions. The resulting encapsulates are analyzed for particle size, gluten vs.
94 hydrogel loading, and release and fibrilization properties in the actual meat-analog
95 production process.

96 # 2 Materials and Methods

97 ## 2.1 Materials

98 All materials were used without further purification, unless stated otherwise. A blend
99 of soy protein isolate (SPI) (SUPRO EX37 HG IP, Solae, USA) and vital wheat

gluten (WG) (VITEN, Roquette, France) was used. In the case of SPI, we determined a protein content of 90 % w/w, while gluten had a protein content of 81 % w/w based on a nitrogen-to-protein conversion factor of 6.25, measured with the Dumas method. Sodium chloride, referred to as salt hereafter, was also used. Alginic acid sodium salt from brown algae, $CaCl \cdot 2H_2O$ (≥ 99%), κ-carrageenan – sulfated plant polysaccharide – and KCl (≥ 99.0%) were purchased from Sigma Aldrich. Peanut oil was purchased from a local supermarket; the oil was colored red using a food-grade dye (a mixture of E-numbers E110 sunset yellow FCF, E122 azorubin, E132 indigontine and E151 Brilliant Black BN) for visualization purposes.

2.2 Methods

2.2.1 Encapsulator

For the immobilization the Encapsulator B-390 from Büchi Labortechnik was used (Figure 1). The sodium alginate and κ-carrageenan were vigorously mixed with water to form a biopolymer solution (1), into which the gluten were stirred vigorously to form a homogeneous immobilization mixture. The immobilization mixtures were led through a (concentric) nozzle (3), after which jet break-up was achieved by vibrations received from the vibration coil (2). The stroboscope (4), that uses the same frequency as the vibration unit, was used to verify the droplet formation (9). A ring (8) was used for electrostatic dispersion of the droplets. The resulting droplets were gelled at room temperature in a 100mM solution (5) of $CaCl_2$ or KCl for the alginate and κ-carrageenan, respectively. The air pressure for pumping (varied between P = 400 – 800 mbar), vibration frequency (varied between F = 200 – 1,000 Hz), amplitude (varied between A = 5 – 9), nozzle temperature (varied between T_N = RT – 65°C) and electrostatic potential (when used, varied between V = 1,000 – 2,500 V) were

controlled using the control panel on the encapsulator (6). The volumetric flow rate Φ_V (varied between Φ_V = 2 – 20 mL/min) was controlled by both the interplay between the applied air pressure and separate regulating valves for the core and coat liquids.

2.3 Analysis

Microscopy

Different optical microscopes were used for the analysis of the resulting particles. A Leica Nikon Optiphot 200 was used, as well as a Leica S6D. Closer inspection of the particles and the sheared material was done with a scanning electron microscope (SEM), FEI Nova NanoSEM650. The samples were used as-is under low vacuum (100 Pa) conditions under relatively low (4.0 kV) acceleration voltages, without the need for applying a conductive coating on the particles.

Composition

The composition of the particles was checked by determining the amount of water the particles hold after cross-linking and removing the excess cross-linking solution by dabbing with a paper towel. Care was taken to minimize the contact of the particles with the paper towels, to minimize the water removal from the inside of the spheres. Samples were weighed before and after drying, from which the water content was calculated. The dry mass was assumed to have the same mass ratio of gluten and hydrogel as initially used before cross-linking.

Melting

The melting behavior of the particles was assessed using the Crystalline multiple reactor system (Avantium B.V.). The particles were loaded in a vial until the top layer of particles was visible in the camera. The vial was heated to 95°C with a heating rate of 0.3°C/min, pictures were taken every 30 s.

Mechanical properties

2.3.1 Static stress scans were performed with a PerkinElmer Dynamic Mechanical Analyzer (DMA) 7e with parallel plate geometry, using a range of 0 – 1,000 mN and a rate of 100 mN/min. The deformation tests were carried out on two different types of particles: Alginate particles loaded with WG, and κ-carrageenan particles loaded with WG. The alginate particles had a diameter of 3 mm. The κ-carrageenan was measured at two different sizes: 3 mm and 1 mm . Thus, the cross sectional area relevant for calculating the normal stress was 7.07 mm^2 for particles with a diameter of 3 mm and 0.79 mm^2 for particles with a diameter of 1 mm. The force, distance and stress were recorded by the DMA software. The bead diameter was entered in the software as diameter for the stress and strain calculations. However, the particle diameter is much smaller than the top cylinder (10 mm) and bottom plate (20 mm) of the DMA. This means that the numerical values from the equipment did not represent the true modulus of the materials and the results from the compression tests could only be compared to each other. Couette Cell

The release behavior of the particles and fibrilizing capabilities of the released gluten at process conditions were tested in the Couette Cell, with the same operating conditions as used in Krintiras et al.(Krintiras et al., 2015). The gluten encapsulates were, after removal of excess cross-linking solution with a paper towel, partially dried in an oven prior to the preparation of the shearing mixture, to obtain a water-gluten ratio close to that used in experiments without encapsulates. First the meat analog

mixture was prepared by mixing 150 g of partially dried encapsulates with 46 g of SPI and 0.5 g of salt, which accounts for the amount of salt in the biopolymer, carefully with a spoon. This mixture was covered and set to rest for 30 minutes, similar to experiments without particles(Krintiras et al., 2015), and then loaded into the Couette Cell.

3 Results and Discussion

For the optimization of the encapsulate production, first the production of spherical beads of WG loaded hydrogel was optimized. Subsequently, the resulting spherical encapsulates were tested on their performance.

3.1 Particle production

The production of alginate particles containing WG was straightforward using the encapsulator. Sodium alginate – WG – water mixtures were led through the single nozzle configuration and cross-linked in a bath containing a $CaCl_2$ solution. The flow rate and the vibration frequency were optimized for each nozzle diameter. A sodium alginate concentration in water of 0.8 % w/w was used. Higher concentrations of sodium alginate in the starting mixture made the mixture more difficult to pump through the nozzle due to increasing viscosity. Additionally, the cross-linked spheres were stronger when higher concentrations of sodium alginate were used, which is undesirable, since too strong particles do not break under the processing conditions. Using lower concentrations of sodium alginate in the starting mixture eventually led to droplets that were mechanically too weak. These droplets disintegrated upon impact with the cross-linking bath and did not produce any microspheres. The settings required for bead formation depended on the mixture and the nozzle used. For example, forming bead with 0.8 % w/w alginate and 3.7 % w/w gluten in water

198 through a nozzle with a diameter of D_N = 750 µm required a pressure, flow rate and
199 vibration frequency of 456 mbar, 9.8 mL/min and 200 Hz, respectively. In Figure 2
200 (a) particles are shown of which the immobilization mixture consisted of 1.65 % w/w
201 alginate and 1.5 % w/w gluten. The gluten is clearly visible in the hydrogel, though
202 not evenly distributed. In Figure 2 (b) the immobilization mixture consisted of 1.65 %
203 w/w alginate and 3.5 % w/w gluten. The gluten in this particle is packed much more
204 dense than in Figure 2 (a), though the distribution of the gluten inside the particles is
205 not clearly visible anymore.
206
207 For the production of κ-carrageenan particles different settings were required.
208 Mixtures containing 2% κ-carrageenan in water were used. Because of the gelling
209 temperature of the κ-carrageenan solution (42°C for 2% solution (Ogbonna, 2004)),
210 the immobilization mixture was heated to 60°C to facilitate the flow to the nozzle.
211 With the bead production in the single nozzle configuration and nozzle heating at
212 T_N = 50°C, the jet break-up occurred at a larger distance from the nozzle than with the
213 alginate particles. Additionally, the particles were not spherical after gelling and not
214 always separated. This is attributed to the droplets losing their spherical shape upon
215 impact with the water or to the long time required for gelling.
216 The bead formation was optimized first for κ-carrageenan without WG. Several
217 configurations were used to increase the sphericity of the particles, which is beneficial
218 for the flow behavior and therefore aids the loading step.
219 Keppeler et al. (Keppeler et al., 2009) found that dripping the droplets through a layer
220 of oil on top of the gelling bath helped the particles attain a spherical shape. Such a
221 layer was used and additionally it was decided to further employ this feature of oil by
222 using the concentric nozzle configuration and using oil in the outer nozzle around the

223 immobilization mixture in the inner nozzle. Figure 3 illustrates this configuration.
224 During the experiment, the thickness of the layer of oil on top of the bath increased
225 due to the addition of the oil via the concentric nozzle. The oil separated from the
226 particles after immersion in the gelling bath and floated to join the oil layer already
227 present, making it easy to separate and reuse. After gelling, the particles were filtered
228 from the salt solution and then washed with demineralized water to remove the oil
229 residues. In this configuration the strength of the spheres was optimized by using
230 lower concentrations of κ-carrageenan. However, at a concentration of 1 % w/w no
231 particles could be made and particles resulting from a 1.5 % w/w solution were
232 mechanically very weak. Therefore, a 2 % w/w solution was considered to provide
233 encapsulates of an acceptable mechanical strength.
234 An example of the optimum mixture (2 % w/w κ-carrageenan and 7 % w/w gluten) is
235 shown in Figure 2 (c). Settings for the optimum mixture were: $T_N = 60°C$,
236 $F_{vib} = 200$ Hz, P = 757 mbar, $D_{NI} = 750$ µm, $D_{NO} = 900$ µm, with flow rates of the κ-
237 carrageenan-gluten mixture $\Phi_{cg} = 6.25$ mL/min and of oil $\Phi_{oil} = 5$ mL/min. The
238 particle size was $d_p = 1.50 \pm 0.23 \cdot 10^3$ µm taken from six separate experiments.

239 ## 3.2 Evaluation of particle properties

240 The suitability of the produced particles to release the encapsulated gluten as a result
241 of shear and elevated temperature in the Couette Cell was assessed by various
242 parameters: the composition in the particles and the behavior of the particles under
243 influence of increasing temperature, simple shear and compression forces were
244 investigated. From the material with the most desirable properties the behavior was
245 also tested in the shear cell. Because the hydrogels can swell in an aqueous
246 environment, the composition of the particles was checked by determining the amount
247 of water the particles hold after gelling (as opposed to the initial concentrations used)

and removing the excess gelling solution by dabbing with a paper towel. Care was taken to minimize the time of contact of the particles with the paper towels, in order to avoid removing water from the inside structure of the spheres. Table 1 shows the composition of a selection of particles.

While the particles containing WG have a water content similar to that of the initial immobilization mixture, the results in Table 1 show that some particles had a slightly lower water content than expected from the initial hydrogel concentration used in the immobilization mixture. It is likely that the drying using the paper towel removed more liquid than just the excess gelling solution. Due to the porous structures of the biopolymer particles, it is possible that a small amount of water was subtracted from the inner structure. The amount of water taken from the particles during the removal of excess water with the paper towels is considered very minimal, since the difference between expected and measured water loading is less than 1%. It was observed that the effect was stronger in particles without WG, as opposed to particles with WG. This indicates that the WG helps the hydrogel to retain the water in its structure, which is expected since gluten is well known to bind water (Day et al., 2006, Sarkki, 1979, Xue and Ngadi, 2007).

The particles were subjected to a temperature profile to assess the behavior upon heating. In Figure 4 κ-carrageenan particles with gluten were heated to 95°C with a heating rate of 0.3°C/min. At T = 20°C the individual particles on top are clearly visible in the circles. Around T = 40°C the surface is changing shape, indicating that the particles started melting. It is well known that κ-carrageenan forms a thermo-reversible gel with water and cations and can thus be melted (Mangione et al., 2003,

Meunier et al., 2001, Guiseley, 1989). This agrees with results from Watase et al. (Watase and Nishinari, 1987) and Nishinari et al. (Nishinari et al., 1990), who found with DSC studies that κ-carrageenan in lower concentrations (1.5 – 2 %w/w) melts above 40°C. Upon increasing the temperature even further, the deformation of the meniscus between particles and air increased, until a flat profile was observed at T = 68°C and the particles were completely molten. This means that at the intended processing temperature of 95°C the particles will melt and release the gluten from their structure. With the alginate particles this was not the case. These particles remained intact up to T = 95°C and showed no change in shape, which is in good agreement with earlier research stating that alginate gel is not thermo-reversible (Guiseley, 1989, Williams et al., 2004). This means that the particles would not release the gluten at the intended processing temperature without mechanical action. Additionally, the particles were compared on their capability to deform under compressive stress. In Figure 5 the deformation of the particle is plotted as the stress - strain curve, resulting from the compressive force applied to the particles by the upper cylinder of the DMA. These deformation tests were carried out on two different types of particles: Alginate particles loaded with WG, and κ-carrageenan particles loaded with WG. The alginate particles had a diameter of 3 mm. The κ-carrageenan was measured at two different sizes: 3 mm and 1 mm diameter, to assess both the influence of particle size and type of hydrogel used. In Figure 5 it is observed that all particles show an elastic behavior and particularly the alginate particles loaded with WG. In the case of the κ-carrageenan particles we observed a shorter elastic region followed by a larger plastic region. The three-dimensional structure of alginate is cross-linked with ionic bonds, while the structure of κ-carrageenan exists of helices. This explains why the elastic region is larger in the case of the alginate hydrogel as

298 opposed to that of κ-carrageenan, since the elastic strains are mainly due to uncoiling
299 and stretching of the structure, while the plastic deformation is caused by molecular
300 chains sliding along each other. The latter phenomenon is easier to achieve with
301 helical structures than cross-linked ones, since the cross-links provide anchors that
302 prevent the chains from moving past one another. We also observed that larger
303 particles require more force for the deformation. This can be caused either by the ratio
304 of pore size versus particle size, or by the amount of mass to be compressed. It was
305 observed that after compression a puddle of water surrounds the particle. During the
306 deformation the water contained in the particles exits through the pores of the
307 hydrogel. The pore size of the hydrogel is assumed to be independent of the particle
308 size. The larger specific area of the pores in the smaller particles is assumed to allow
309 for easier expulsion of the water and is therefore associated with a smaller
310 compressive stress.
311
312
313 **Couette Cell**
314 In other work (Krintiras et al., 2014, Krintiras et al., 2015) the Couette Cell was used
315 with free gluten powder. The sheared mixtures had a composition like that in Table 2,
316 but without the hydrogel component and with more salt. In their work, the fibrous
317 structure on both micro and macroscale are clearly visible.
318 Both the alginate and the κ-carrageenan particles were tested in the Couette Cell to
319 assess whether fiber formation occurs after release of the gluten from their
320 encapsulated environment. Before shearing, the shearing mixture was prepared. The
321 encapsulates were, after removal of excess cross-linking solution, partially dried in an
322 oven, until they contained 88 ± 1 % w/w of water, giving a similar water/gluten ratio

323 as the shearing composition. Subsequently, they were mixed with SPI and salt to
324 arrive at a final composition given in Table 2.
325
326 Figure 6 shows the preparation of the shearing mixture using the particles. The
327 particles were mixed with the SPI and salt and left to rest (a). It was observed that the
328 soy coated both the alginate-gluten and the κ-carrageenan-gluten particles and
329 hydrated by subtracting water from the particles during the resting period (b). The
330 level of hydration of the soy seemed similar to when free water is used.
331
332 After preparation of the mixture, it was tested how well the mixture loads in the
333 Couette Cell using the loading gun. The loading procedure was completed without
334 complications and the mixture spread well throughout the Couette Cell. Many of the
335 particles were still intact, although some had been broken. Closer inspection of the
336 material showed no evidence of fibrilization at this stage.
337 Figure 7(a) shows the alginate-gluten sample after shearing in the Couette Cell.
338 Throughout the sample the particles were still visible and albeit deformed, they were
339 still intact. Microscope images of the material revealed that very limited fibrilization
340 occurred, and only on or surrounding the particles, but nowhere else in the structure.
341 From the entire sample it was also evident that no macrostructure developed. The
342 material fell apart upon movement, since the particles provided break lines in the
343 sample.
344
345 Figure 7(b) shows a sheared sample of κ-carrageenan-gluten particles directly after it
346 was taken from the Couette Cell. No separate particles are visible. All particles had
347 released their gluten and the biopolymer was homogeneously mixed through the

sample. The sample did not fall apart like its alginate counterpart, indicating that the macrostructure was more developed. Microscope images of this sample showed numerous gluten fibers throughout the sample.

SEM pictures of the sheared κ-carrageenan sample (Figure 8) confirm the observations with the optical microscope. In Figure 8(a) a larger part of the sample is shown with three of the fibers sticking out of the material. In Figure 8(b), the three types of material are visible: the gluten fiber, the soy (1) and the surrounding hydrogel (2). The materials were mixed well throughout the sample. The fibers show a wrinkly surface structure, which is also clearly visible in Figure 8(b). This structure is due to the hierarchical nature of the fibers, i.e. the fibers are made up out of smaller fibrils, which was earlier shown for gluten by Ridgley et al. (Ridgley et al., 2012). Changing processing parameters, like temperature, ionic strength and shearing time can influence both the extent of fiber formation, as well as the structure formation. For different sets of processing parameters different structures (e.g. ribbons) were found (Ridgley et al., 2012). It was also observed that the fibers seem to be built up layer by layer from the fibrils, which is most clearly evident from Figure 8(b) in circle 3. The gluten fibers had various diameters. Larger and smaller fibers were observed next to each other, the larger having diameters of 20 ± 3 μm, the smaller 13 ± 2 μm.

Discussion

When immobilizing or encapsulating, the choice of encapsulant is very important. Not only the processing, but also the final composition of the product materials must meet requirements in terms of process conditions and product quality. In the food sector, additional requirements need to be met, which in our case are that the encapsulant is food-grade material and does not alter the ingredient mixture or taste by a significant extent. Requirements for the final product include that the final product is easy to use

373 in the shearing process. This would benefit from spherical particles to make the
374 mixture mix and load easily. These requirements led to our choices of hydrogels,
375 which are easy to process, food-grade materials and tasteless (Burdock, 1997, 2006).
376 The dripping technique employed by the encapsulator is particularly suitable for these
377 materials (Mazzitelli et al., 2008, Matalanis et al., 2011, Danial et al., 2010), since it
378 easily leads to spherical particles.
379 Judging by the melting and compression behavior of the particles it was expected that
380 the κ-carrageenan particles would show better controlled-release properties than the
381 alginate particles, while at room temperature each of them can prevent the gluten from
382 cross-linking.
383 From the results it is clear that the hydrogels used are very well capable of
384 immobilizing the gluten in aqueous environments. The controlled release of the gluten
385 by increased temperature and shear, however, was more easily achieved from the
386 κ-carrageenan particles than from the alginate particles. In the Couette Cell this
387 behavior was confirmed. The alginate particles are so strong that they do not break or
388 dissolve under the preferred process conditions and thus do not release their gluten for
389 fibrilization. The very limited amount of fibers observed in the sheared sample
390 containing alginate, together with the location of these fibers, i.e. only on top of, or
391 very close to the unbroken particles, are a clear indication that this immobilization
392 material is too strong for the purpose. The κ-carrageenan particles did release the
393 gluten and fibrilization occurred to a much larger extent during the shearing process.
394 However, while comparing the structure sheared from the particles with the structures
395 obtained after shearing the original mixture without particles (Krintiras et al., 2014,
396 Krintiras et al., 2015), it was observed that although fibrilization occurs, it is much
397 less than with the original mixture. The macrostructure of the meat analog is not yet

well developed. However, both samples were sheared with the settings optimized for the original mixture. The particles took a long time to melt and release their content when the temperature is increased, which was evident from the melting test in Figure 4. Therefore, it is likely that the shear time must be increased, or that a preheating step must be added to allow for the particles to soften prior to shearing. Additionally, the mechanical properties of the particles determine in part the optimum processing in the Couette Cell. Measurement and understanding of these particle properties as function of water content as well as of the Couette Cell operating conditions is imperative in the future optimization of the structuring process. Finally, the 2 % w/w of κ-carrageenan interacts with the mixture, as is also seen in Figure 8(b), where the fiber in the picture is partially surrounded with the hydrogel. It is possible that the hydrogel surrounding the fibers actually inhibits the formation of 3D-structures required for a desirable meat analog. Prior to application of immobilized gluten in meat analogs, the settings of the shearing process should be optimized for the new materials used, and the effect of the hydrogel on the mouthfeel of the final meat analog should be assessed.

The successful production of spherical particles of κ-carrageenan with the aid of oil shows that the dripping technique can be used for a wide variety of applications that require the production of spherical encapsulates. For applications such as the immobilization of vitamins, fragrance and pharmaceutical ingredients, the hydrogels are a very suitable encapsulant. However, in other industries the same dripping technique can be used with many other polymers as well, leading to other coating functionalities, e.g. protection from oxygen or moisture from the air. As long as the polymer in question has a low enough melting temperature or suitable cross-linking conditions, the dripping method can be used. The technique is easily scalable to larger

capacity by using an array of nozzles. In the case that the vibrations are not sufficient to achieve the jet break-up, other jet break-up techniques (e.g. jet cutting) could be used instead. For continuous operation a cascaded hardening bath can be used, in which the desired residence time can be achieved.

4 Conclusion

In this paper Wheat Gluten is successfully encapsulated in a matrix of a food-grade biopolymer. Both sodium alginate and κ-carrageenan were used as encapsulants. While the particle properties of κ-carrageenan surpassed those of alginate, the particle production was more complicated. In order to obtain a good sphericity of the particles, with κ-carrageenan it was required to use a layer of oil on the gelling bath, as well as through the concentric nozzle. For the alginate particles no oil phase was required. In the alginate particles a loading of 7 % w/w gluten was achieved in the particles with 1.5 % w/w alginate. Controlled release of the gluten from the alginate particles was not achieved properly by temperature or shear. In κ-carrageenan, a loading of 7 % w/w gluten was achieved in the particles, next to 2 % w/w of κ-carrageenan. Lower amounts of κ-carrageenan did not lead to separate, spherical particles. The water content of the particles can be easily controlled by a subsequent partial drying step. The controlled release of the gluten was achieved at the processing conditions only with κ-carrageenan. Some fibrilization was observed in the sheared product. However, the shearing process needs to be optimized for the use of the particles to obtain a good structure for the meat analog. The technique used for the immobilization of gluten shows promise for the immobilization or protection of other core materials, in the food industry as well as in other industries, where the food grade

446 biopolymers can be replaced by any polymer with an acceptable melting temperature
447 or cross-linking conditions.

448 ## Bibliography

449 2006. Remington: The Science and Practice of Pharmacy, Lippincott Williams &
450 Wilkins.ISBN: 0781746736
451 ABANG ZAIDEL, D. N., CHIN, N. L., ABDUL RAHMAN, R. and KARIM, R.
452 2008. Rheological characterisation of gluten from extensibility measurement.
453 Journal of Food Engineering, **86,** 549-556.
454 http://dx.doi.org/10.1016/j.jfoodeng.2007.11.005
455 BAI, C., ZHANG, S., HUANG, L., WANG, H., WANG, W. and YE, Q. 2015.
456 Starch-based hydrogel loading with carbendazim for controlled-release and
457 water absorption. Carbohydrate Polymers, **125,** 376-383.
458 http://dx.doi.org/10.1016/j.carbpol.2015.03.004
459 BURDOCK, G. A. 1997. Encyclopedia of Food and Color Additives, CRC Press.
460 ISBN: 0-8493-9416-3
461 DANIAL, E. N., ELNASHAR, M. M. M. and AWAD, G. E. A. 2010. Immobilized
462 Inulinase on Grafted Alginate Beads Prepared by the One-Step and the Two-
463 Steps Methods. Industrial & Engineering Chemistry Research, **49,** 3120-3125.
464 http://dx.doi.org/10.1021/ie100011z
465 DAY, L., AUGUSTIN, M. A., BATEY, I. L. and WRIGLEY, C. W. 2006. Wheat-
466 gluten uses and industry needs. Trends in Food Science & Technology, **17,** 82-
467 90.
468 DOHERTY, S. B., GEE, V. L., ROSS, R. P., STANTON, C., FITZGERALD, G. F.
469 and BRODKORB, A. 2011. Development and characterisation of whey
470 protein micro-beads as potential matrices for probiotic protection. Food
471 Hydrocolloids, **25,** 1604-1617.
472 http://dx.doi.org/10.1016/j.foodhyd.2010.12.012
473 ELZOGHBY, A. O., ABO EL-FOTOH, W. S. and ELGINDY, N. A. 2011. Casein-
474 based formulations as promising controlled release drug delivery systems.
475 Journal of Controlled Release, **153,** 206-216.
476 http://dx.doi.org/10.1016/j.jconrel.2011.02.010
477 GUISELEY, K. B. 1989. Chemical and physical properties of algal polysaccharides
478 used for cell immobilization. Enzyme and Microbial Technology, **11,** 706-716.
479 HOEK, A. C., LUNING, P. A., WEIJZEN, P., ENGELS, W., KOK, F. J. and DE
480 GRAAF, C. 2011. Replacement of meat by meat substitutes. A survey on
481 person- and product-related factors in consumer acceptance. Appetite, **56,** 662-
482 673. http://dx.doi.org/10.1016/j.appet.2011.02.001
483 HUGHES, D. 1995. Animal welfare. British Food Journal, **97,** 3-7.
484 http://dx.doi.org/10.1108/00070709510104529
485 KEPPELER, S., ELLIS, A. and JACQUIER, J. C. 2009. Cross-linked carrageenan
486 beads for controlled release delivery systems. Carbohydrate Polymers, **78,**
487 973-977. http://dx.doi.org/10.1016/j.carbpol.2009.07.029
488 KRINTIRAS, G. A., GOBEL, J., BOUWMAN, W. G., JAN VAN DER GOOT, A.
489 and STEFANIDIS, G. D. 2014. On characterization of anisotropic plant
490 protein structures. Food & Function, **5,** 3233-3240.

KRINTIRAS, G. A., GÖBEL, J., VAN DER GOOT, A. J. and STEFANIDIS, G. D. 2015. Production of structured soy-based meat analogues using simple shear and heat in a Couette Cell. Journal of Food Engineering, **160,** 34-41. http://dx.doi.org/10.1016/j.jfoodeng.2015.02.015

LI, R., SHU, C., WANG, W., WANG, X., LI, H., XU, D. and ZHONG, W. 2015. Encapsulation of 10-Hydroxy Camptothecin in Supramolecular Hydrogel as an Injectable Drug Delivery System. Journal of Pharmaceutical Sciences, **104,** 2266-2275. http://dx.doi.org/10.1002/jps.24481

MA, G. 2014. Microencapsulation of protein drugs for drug delivery: Strategy, preparation, and applications. Journal of Controlled Release, **193,** 324-340. http://dx.doi.org/10.1016/j.jconrel.2014.09.003

MANGIONE, M. R., GIACOMAZZA, D., BULONE, D., MARTORANA, V. and SAN BIAGIO, P. L. 2003. Thermoreversible gelation of κ-Carrageenan: relation between conformational transition and aggregation. Biophysical Chemistry, **104,** 95-105.

MATALANIS, A., JONES, O. G. and MCCLEMENTS, D. J. 2011. Structured biopolymer-based delivery systems for encapsulation, protection, and release of lipophilic compounds. Food Hydrocolloids, **25,** 1865-1880. http://dx.doi.org/10.1016/j.foodhyd.2011.04.014

MAZZITELLI, S., TOSI, A., BALESTRA, C., NASTRUZZI, C., LUCA, G., MANCUSO, F., CALAFIORE, R. and CALVITTI, M. 2008. Production and Characterization of Alginate Microcapsules Produced by a Vibrational Encapsulation Device. Journal of Biomaterials Applications, **23,** 123-145. http://dx.doi.org/10.1177/0885328207084958

MEUNIER, V., NICOLAI, T. and DURAND, D. 2001. Structure of aggregating κ-carrageenan fractions studied by light scattering. International Journal of Biological Macromolecules, **28,** 157-165.

NISHINARI, K., WATASE, M., WILLIAMS, P. A. and PHILLIPS, G. O. 1990. .kappa.-Carrageenan gels: effect of sucrose, glucose, urea, and guanidine hydrochloride on the rheological and thermal properties. Journal of Agricultural and Food Chemistry, **38,** 1188-1193.

OGBONNA, J. 2004. Atomisation Techniques for Immobilisation of Cells in Micro Gel Beads. In Fundamentals of Cell Immobilisation Biotechnology, (NEDOVIĆ, V. & WILLAERT, R., eds.) pp. 327-341, Springer Netherlands. http://dx.doi.org/10.1007/978-94-017-1638-3_18

ORIVE, G., HERNÁNDEZ, R., GASCÓN, A. and PEDRAZ, J. 2006. Encapsulation of Cells in Alginate Gels. In Immobilization of Enzymes and Cells, (GUISAN, J., ed.) pp. 345-355, Humana Press. http://dx.doi.org/10.1007/978-1-59745-053-9_30

ORIVE, G., HERNANDEZ, R. M., GASCON, A. R., CALAFIORE, R., CHANG, T. M. S., VOS, P. D., HORTELANO, G., HUNKELER, D., LACIK, I., SHAPIRO, A. M. J. and PEDRAZ, J. L. 2003. Cell encapsulation: Promise and progress. Nat Med, **9,** 104-107. http://dx.doi.org/10.1038/nm0103-104

PAPAGEORGIOU, M., KASAPIS, S. and GOTHARD, M. G. 1994. Structural and textural properties of calcium induced, hot-made alginate gels. Carbohydrate Polymers, **24**, 199-207.

RIDGLEY, D. M., CLAUNCH, E. C. and BARONE, J. R. 2012. The effect of processing on large, self-assembled amyloid fibers. Soft Matter, **8,** 10298-10306. http://dx.doi.org/10.1039/C2SM26496J

SARKKI, M.-L. 1979. Food uses of wheat gluten. Journal of the American Oil *Chemists' Society,* **56,** 443-446.

STEINFELD, H., FOOD, AGRICULTURE ORGANIZATION OF THE UNITED, N., LIVESTOCK, E. and DEVELOPMENT. 2006. Livestock's long shadow : environmental issues and options, Food and Agriculture Organization of the United Nations, Rome. ISBN: 9789251055717

TECANTE, A. and SANTIAGO, M. D. C. N. 2012. Solution Properties of κ-Carrageenan and Its Interaction with Other Polysaccharides in Aqueous Media In Rheology, (VICENTE, D. J. D., ed.), InTech. http://dx.doi.org/10.5772/36619

WATASE, M. and NISHINARI, K. 1987. Rheological and thermal properties of carrageenan gels. Effect of sulfate content. Die Makromolekulare Chemie, **188,** 2213-2221.

WIELAND-BERGHAUSEN, S., SCHOTE, U., FREY, M. and SCHMIDT, F. 2002. Comparison of microencapsulation techniques for the water-soluble drugs nitenpyram and clomipramine HCl. Journal of Controlled Release, **85,** 35-43. http://dx.doi.org/10.1016/S0168-3659(02)00269-9

WILLIAMS, P. A., PHILLIPS, G. O. and CHEMISTRY, R. S. O. 2004. Gums and Stabilisers for the Food Industry 12, Royal Society of Chemistry.

XUE, J. and NGADI, M. 2007. Thermal properties of batter systems formulated by combinations of different flours. LWT - Food Science and Technology, **40,** 1459-1465.

ZANDI, M. 2016. Evaluation of the Kinetics of Ascorbic Acid (AA) Release from Alginate-Whey Protein Concentrates (AL-WPC) Microspheres at the Simulated Gastro–Intestinal Condition. Journal of Food Process Engineering**,** n/a-n/a. http://dx.doi.org/10.1111/jfpe.12334

ZHOU, S., DENG, X. and LI, X. 2001. Investigation on a novel core-coated microspheres protein delivery system. Journal of controlled release : official journal of the Controlled Release Society, **75,** 27-36. http://dx.doi.org/10.1016/s0168-3659(01)00379-0

Acknowledgements

Ben Norder of the ChemE department at TU Delft is acknowledged for the compression measurements. Willem Duvalois of the Energetic Materials department at TNO is acknowledged for the SEM pictures. We thank TNO for the funding of the research.

Tables

TABLE 1.
COMPOSITION OF PARTICLES FOR FURTHER TESTING.

Initial hydrogel concentration		Gluten %	Water %	# particles in sample
Alginate %	κ-carrageenan %			
1.50 ± 0.01	0	7.00 ± 0.01	92.0 ± 0.1	124
0	2.00 ± 0.01	7.00 ± 0.01	90.3 ± 0.5	316

TABLE 2.
COMPOSITION OF SHEARING MIXTURES.

Material	% w/w in shearing composition
Water	69.5 ± 1.3
Gluten	7.3 ± 0.1
Alginate / κ-carrageenan	0.83 ± 0.01/1.09 ± 0.02
Soy	23.6 ± 0.27
Salt	0.50 ± 0.02

Figure captions

FIGURE 1.

BÜCHI ENCAPSULATOR IN CORE-SHELL CONFIGURATION, WITH 1. PRESSURE BOTTLES WITH IMMOBILIZATION MIXTURE (YELLOW) AND OIL (RED), 2. VIBRATION COIL, 3. NOZZLE HOLDER, 4. STROBOSCOPE FOR VISUALIZATION, 5. GELLING BATH, 6. CONTROLS, 7. NOZZLE, 8. ELECTROSTATIC DISPERSION UNIT, 9. JET WITH DROPLETS.

FIGURE 2.

ALGINATE PARTICLES (A AND B) AND K-CARRAGEENAN PARTICLES (C) CONTAINING VARIOUS CONCENTRATIONS OF GLUTEN.

FIGURE 3.

LEFT TOP: SCHEMATIC OF SETUP USING OIL IN THE CONCENTRIC NOZZLE AS WELL AS ON THE GELLING BATH. LEFT BOTTOM: CLOSE UP OF THE IMMOBILIZATION MIXTURE AND OIL EMERGING FROM THE CONCENTRIC NOZZLE. RIGHT: DROPLETS ENTERING THE GELLING BATH THROUGH THE LAYER OF OIL, WHICH SEPARATES THE PARTICLES FROM EACH OTHER.

FIGURE 4.

MELTING OF K-CARRAGEENAN-GLUTEN PARTICLES UPON HEATING. THE TWO TOP PARTICLES ARE CIRCLED IN RED IN THE LEFT-MOST PICTURE. THE FLATTENING OF THE MENISCUS INDICATES THE MELTING OF THESE PARTICLES.

FIGURE 5.

STRESS - STRAIN CURVE OF DIFFERENT PARTICLES UNDER INCREASING COMPRESSION FORCE. ALGINATE-WG (BLUE), AND K-CARRAGEENAN-WG (RED) PARTICLES WITH A DIAMETER OF 3 MM AND 1 MM (RED DASHED) WERE MEASURED.

FIGURE 6.

(A) PREPARATION OF SHEARING MIXTURE; (B) HYDRATED SOY ON A K-CARRAGEENAN PARTICLE.

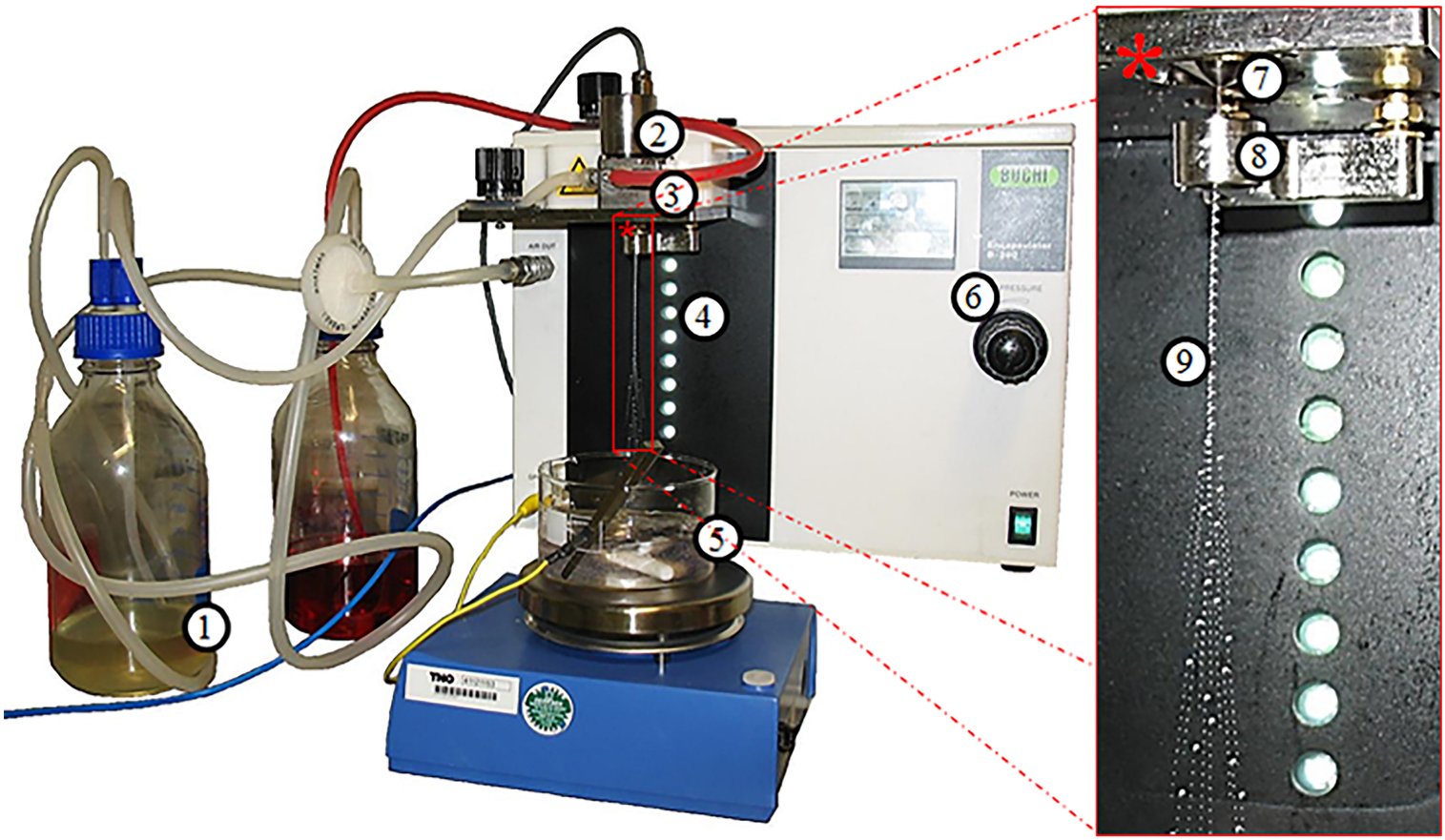
FIGURE 7.

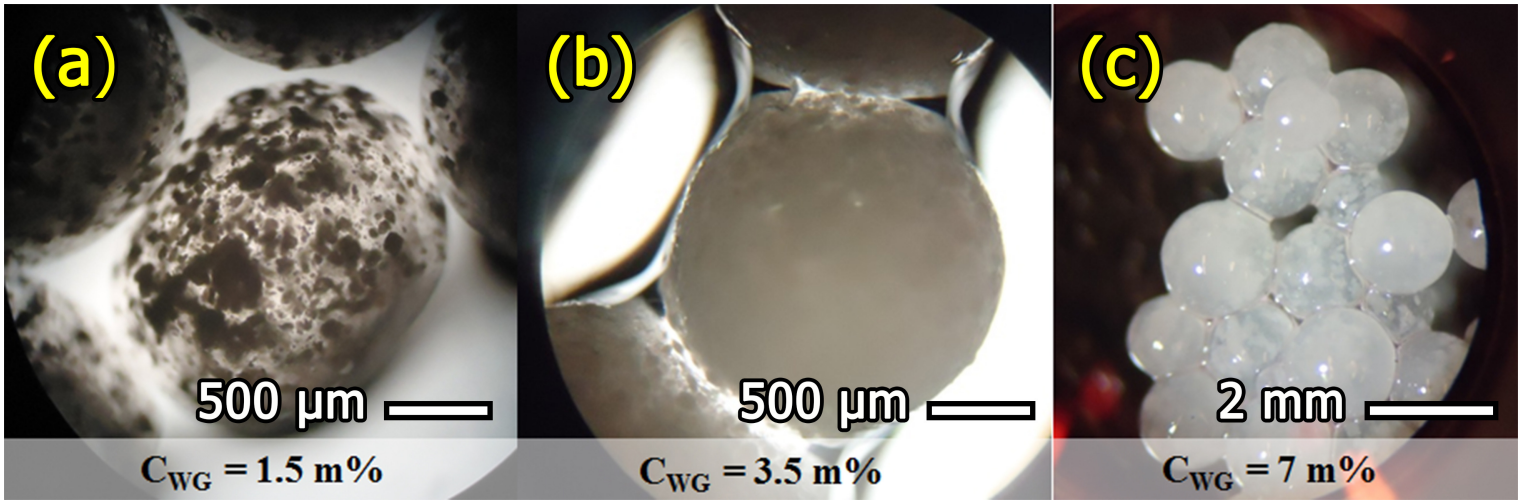
(A) ALGINATE-GLUTEN AFTER SHEARING. MOST OF THE PARTICLES ARE STILL INTACT

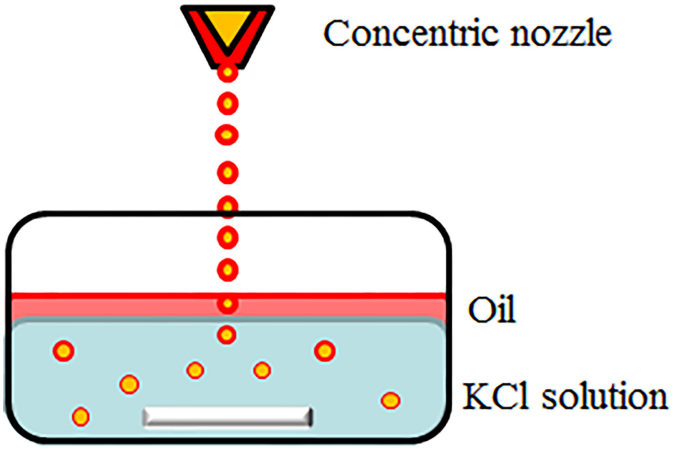
AND THE MACROSTRUCTURE IS NOT WELL DEVELOPED. (B) K-CARRAGEENAN-GLUTEN AFTER SHEARING. NO SEPARATE PARTICLES ARE OBSERVED AND A MORE COHESIVE PRODUCT IS OBTAINED.

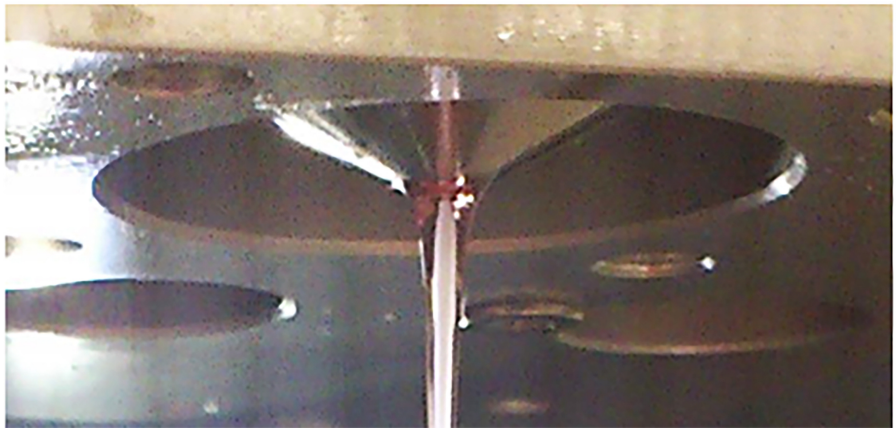
FIGURE 8.

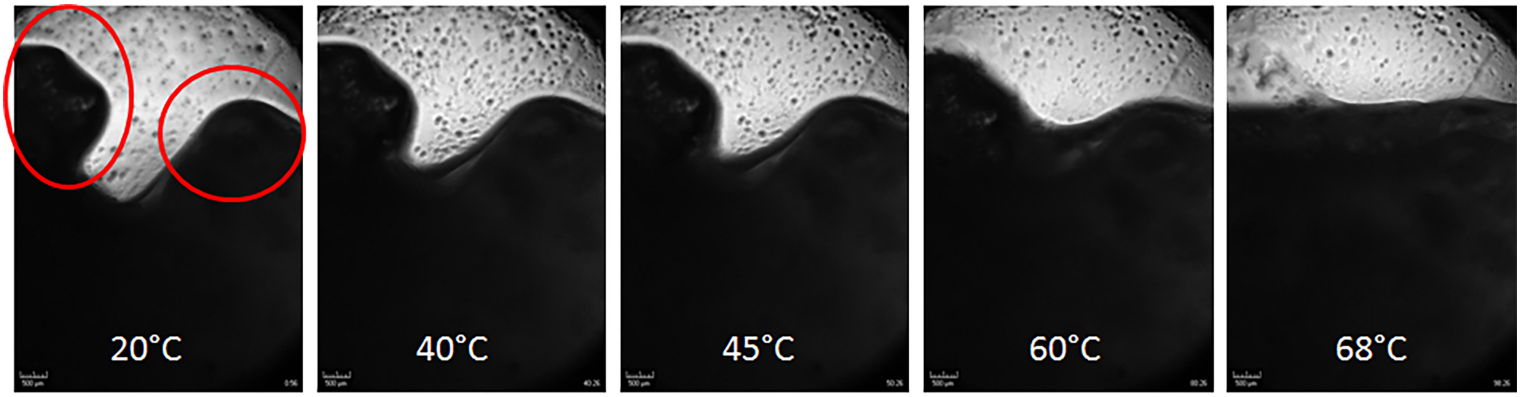
(A) SEM PICTURES AT DIFFERENT MAGNIFICATIONS OF FIBERS IN THE SHEARED SAMPLE WITH PARTICLES OF K-CARRAGEENAN (2% W/W) WITH GLUTEN (7% W/W). MULTIPLE FIBERS WERE OBSERVED. (B) THE SOY (1) AND HYDROGEL (2) ARE VISIBLE NEXT TO THE FIBRIL STRUCTURE AT THE SURFACE OF THE GLUTEN FIBER (3).

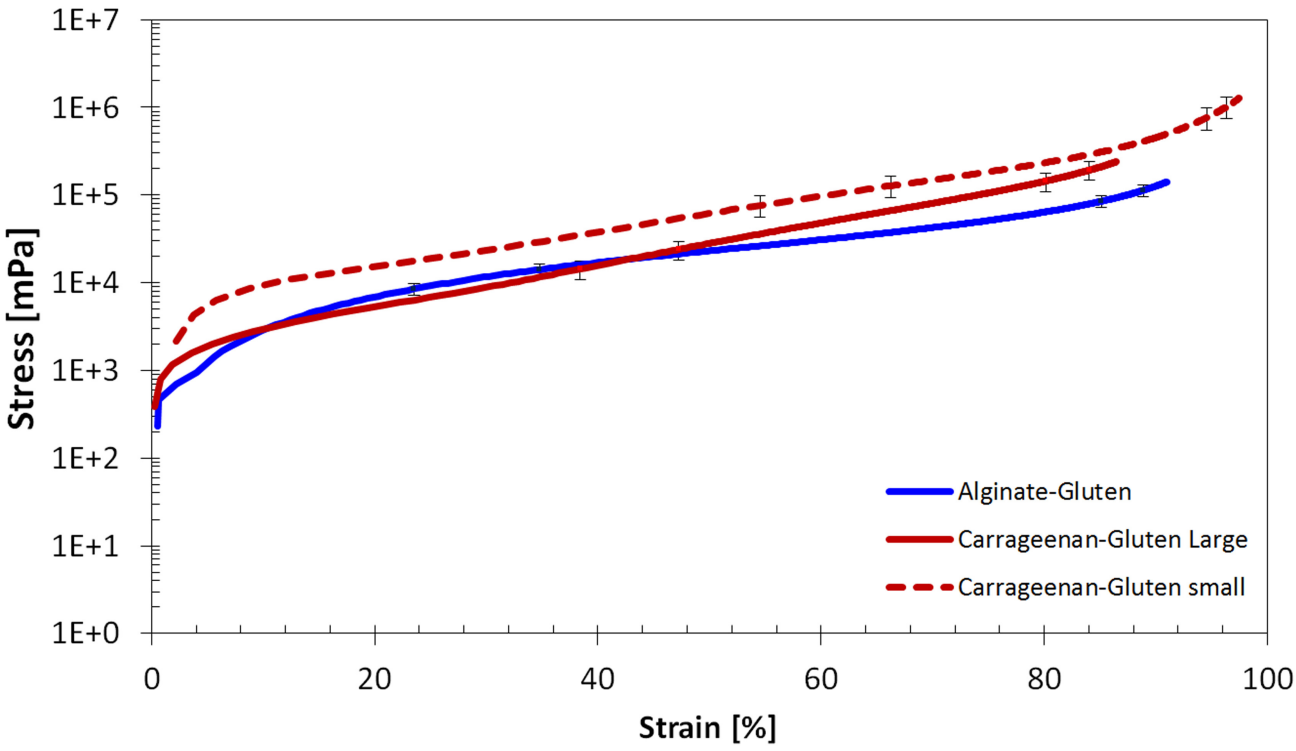

1
2
3
4
5
6
7
8
9
BUCHI

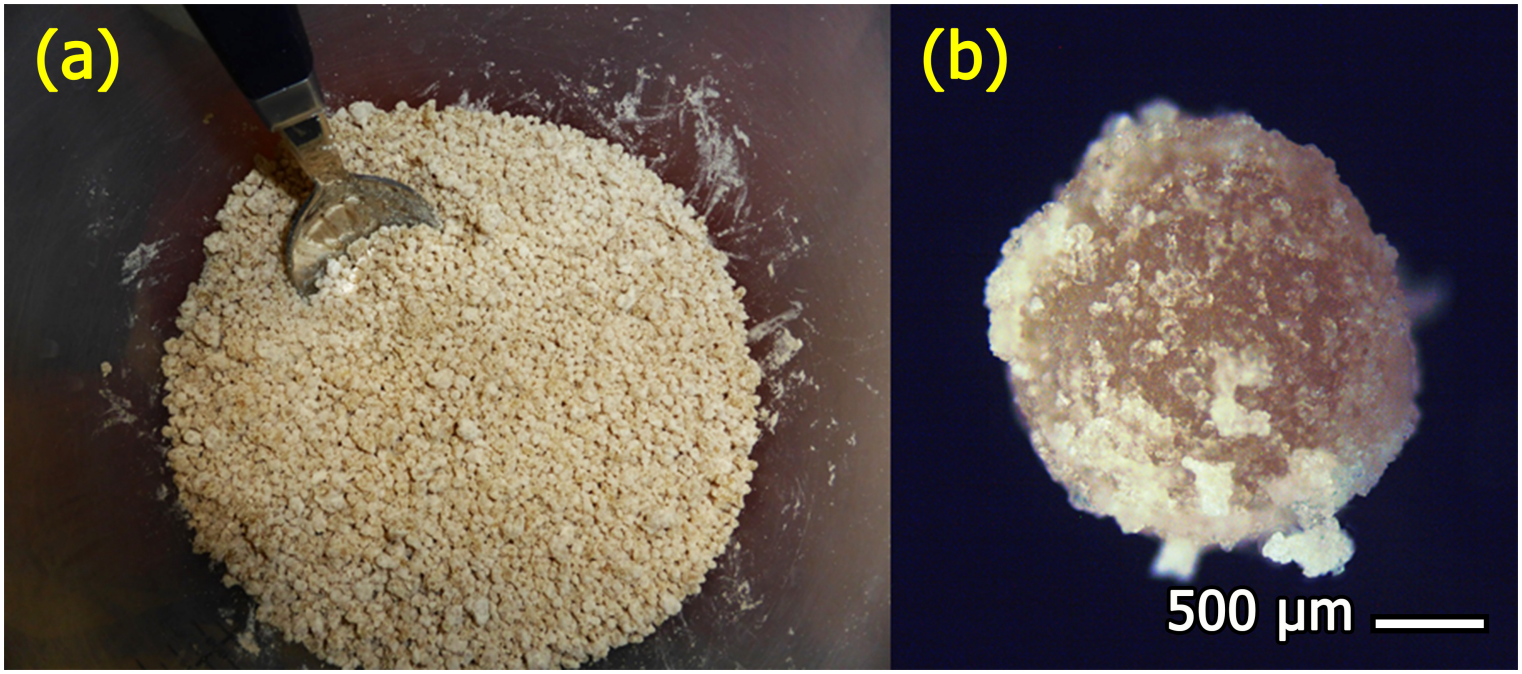

(a)
500 µm
C_{WG} = 1.5 m%
(b)
500 µm
C_{WG} = 3.5 m%
(c)
2 mm
C_{WG} = 7 m%

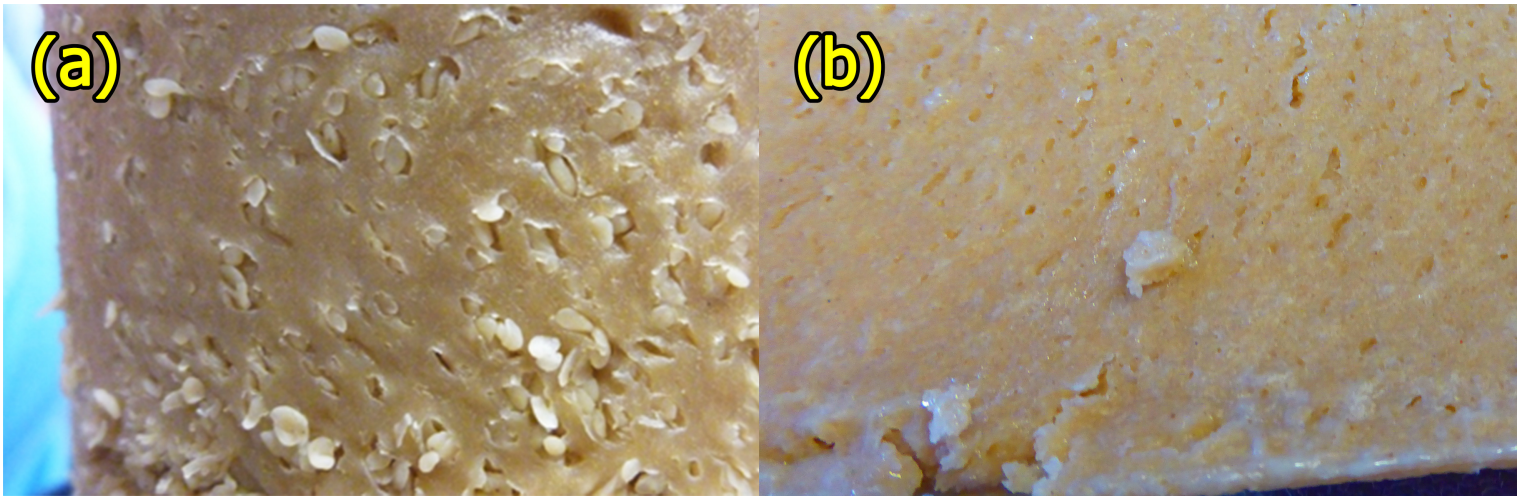

Concentric nozzle
Oil
KCl solution

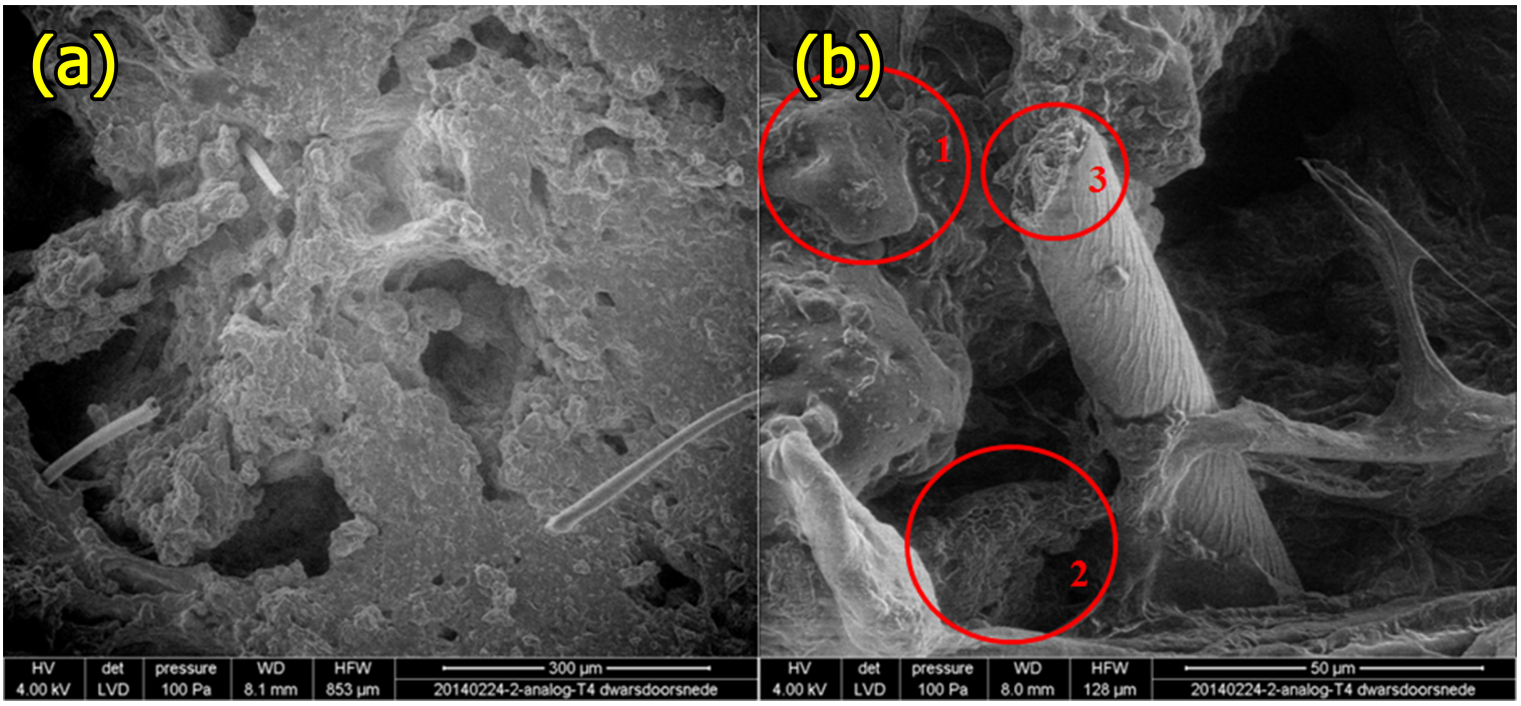



20°C
40°C
45°C
60°C
68°C


1E+7
1E+6
1E+5
1E+4
1E+3
1E+2
1E+1
1E+0
Stress [mPa]
0
20
40
60
80
100
Strain [%]
Alginate-Gluten
Carrageenan-Gluten Large
Carrageenan-Gluten small


(a)
(b)
500 µm


(a)
(b)


(a)
(b)
1
2
3
HV
4.00 kV
det
LVD
pressure
100 Pa
WD
8.1 mm
HFW
853 µm
300 µm
20140224-2-analog-T4 dwarsdoorsnede
HV
4.00 kV
det
LVD
pressure
100 Pa
WD
8.0 mm
HFW
128 µm
50 µm
20140224-2-analog-T4 dwarsdoorsnede