



Strength of microbeads for the encapsulation of heat sensitive, hydrophobic components

Pauline van Leusden, Gertjan J. M. den Hartog, Aalt Bast, Michiel Postema,
Erik van Der Linden, Leonard M.C. Sagis

► To cite this version:

Pauline van Leusden, Gertjan J. M. den Hartog, Aalt Bast, Michiel Postema, Erik van Der Linden, et al.. Strength of microbeads for the encapsulation of heat sensitive, hydrophobic components. Food Hydrocolloids, 2016, 56, pp.318-324. 10.1016/j.foodhyd.2015.12.017 . hal-03192860

HAL Id: hal-03192860

<https://hal.science/hal-03192860>

Submitted on 15 Apr 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0
International License

Strength of microbeads for the encapsulation of heat sensitive, hydrophobic components

Authors: P. van Leusden¹, G.J.M. den Hartog², A. Bast², M. Postema³, E. van der Linden¹,
L.M.C. Sagis¹

*1 Physics and Physical Chemistry of Foods Group, Department of Agrotechnology and Food
Sciences, Wageningen University, Bornse Weiland 9, 6708 WG Wageningen, The
Netherlands*

*2 Department of Pharmacology and Toxicology, Maastricht University, PO Box 616, 6200
MD Maastricht, The, Netherlands*

*3 Department of Physics and Technology, University of Bergen, Allégaten 55, 5007 Bergen,
Norway*

Abstract

Hydrophobic food ingredients sensitive to degradation can be protected from their environment by microencapsulation. In an O/W₁/W₂ system, these hydrophobic compounds are dissolved in oil droplets, dispersed within a gelled matrix microbead (W₁), which forms a barrier. The stability and degree of protection delivered by the gel matrix depends on its structure and strength, which in turn depend on the gelling process. For heat sensitive ingredients this process is typically a cold-set gelling process.

We investigated the effect of variations in matrix polymer (alginate and WPI aggregates), gelling agent (acid and calcium), and method of gelation (internal and external), on the ability

of microbeads to retain oil droplets, and retain a spherical shape during the extraction from the oil phase.

External gelation with CaCl_2 nanoparticles gave the smoothest and strongest microbeads for both protein and alginate, which we attribute to the formation of a shell at the interface of the bead during gelation. Microbeads produced by internal calcium gelation (induced with CaCO_3 nanoparticles and GDL) containing the same amount of calcium showed less integrity and gave a mixture of smooth and rough beads. About half of the microbeads produced by acid induced gelation of WPI aggregates (using GDL to lower the internal pH) remained intact. When the pH was brought further from the isoelectric point, fewer beads remained intact. The method of gelation proved to be more important for the microbead integrity than type of matrix polymer, and external gelling was clearly superior to internal and acid induced gelation.

Keywords: Cold-set gelation, microbeads, encapsulation, strength, gelation methods

1. Introduction

Bioactive components such as vitamins and antioxidants are sensitive components and can be protected from degradation during storage and digestion (Beaulieu, Savoie, Paquin, & Subirade, 2002; Kaushik & Roos, 2007; Yoo, Song, Chang, & Lee, 2006). Protecting these components by encapsulation is a straightforward solution and, if the microcapsules are kept below 25µm, will not negatively affect sensorial aspects (Tyle, 1993). Many types of microcapsules have been suggested and investigated. McClements presented an overview of the most commonly used systems, which includes the filled hydrogel particle (McClements, 2012). This is an O/W₁/W₂ system, in which the sensitive oil-soluble components are dispersed within a gelled matrix microbead (W₁). This gelled phase acts as a barrier against negative environmental influences.

Proteins are well suited for the encapsulation of bioactive compounds (Gunasekaran, Ko, & Xiao, 2007); (Beaulieu, et al., 2002) because they are safe for consumption, have a high nutritional value and are able to form gels and emulsions. Alginate is a polysaccharide that has already been extensively investigated for use in encapsulation (Gombotz & Wee, 2012; Li, Hu, Du, Xiao, & McClements, 2011; Lupo, Maestro, Porras, Gutiérrez, & González, 2014; Yoo, et al., 2006), including encapsulation of living cells (Allan-Wojtas, Truelstrup Hansen, & Paulson, 2008; Annan, Borza, & Hansen, 2008; Smidsrød & Skjåk-Bræk, 1990). Alginate is safe for consumption and cannot be digested, therefore having no nutritional value.

Walther et al. did a model study in which they investigated a method to form gelled protein droplets in an oil phase, by dripping a protein solution in a heated oil bath (Walther, Walkenström, Hermansson, Fischer, & Windhab, 2002). This concept was further developed into a method to create high-protein microparticles by emulsifying a concentrated protein solution into oil, and then inducing gelation by applying heat (Sağlam, Venema, de Vries,

Sagis, & van der Linden, 2011). For protein microparticles, the effects of protein source, including the cold-setting gelatin, and the pH sensitivity of the microparticles was investigated (Ince Coskun, Saglam, Venema, van der Linden, & Scholten, 2015).

The most conventional method to create alginate microparticles is by dripping an alginate solution into a bath with calcium ions, however this method gives microparticles much larger than 25µm (Paques, van der Linden, van Rijn, & Sagis, 2014). Paques et al. showed that a method similar to the method of Saglam et al. (2011) could be used to produce alginate microparticles much smaller than 25 µm (Paques, van der Linden, van Rijn, & Sagis, 2012).

For heat sensitive components, the gelation method of the microbeads should be cold-set gelation. Cold set gels can be made with several methods, including acidification, addition of salts, enzymes and pressure. Acid induced gelation is done by decreasing the pH of the solution to a pH where the polyelectrolyte approaches a net zero charge and the molecules start associating. For WPI this is around pH 5.1 (Bryant & McClements, 1998), for alginate this is below 3.5 (Draget, Skjåk Bræk, & Smidsrød, 1994). For WPI gels, adding salts will cause repulsive forces between the protein molecules to be screened, thereby possibly inducing aggregation. All salts screen the charges, however, divalent salts such as Ca^{2+} have an additional effect as they are able to form salt bridges, thereby reducing the amount of salt necessary for gelation (Kuhn & Foegeding, 1991). Salt bridges with Ca^{2+} are the usual and most effective method of gelation for the negatively charged alginate polymers.

The way in which the gelling agent is introduced into the system can affect the microstructure of the gel. Internal gelation is when the gelling agent is present within the matrix, and slowly causes the matrix to gel from within. Sometimes this gelling agent is activated by a trigger, for example, the insoluble CaCO_3 , which dissociates when the pH is lowered below pH 7. External gelation uses a gelation source which is present outside the matrix, and the diffusion

of the gelling agent into the matrix causes gelation. An example of external gelation is the creation of alginate beads by dripping an alginate solution into a solution with calcium ions. These two methods can give differences in the structure, properties, and therefore use of the microbeads. External gelation of alginate beads has been found to give smoother surfaces, smaller pores, slower diffusion and greater matrix strength, even though the amount of Ca^{2+} bridges formed in internal and external gelation was equal (Liu, et al., 2002); (Chan, Lee, & Heng, 2006). Because acid is necessary for the dissociation of the CaCO_3 , it has been suggested that this method may reduce the pH inside the particles to such levels as to be harmful to the encapsulated component. Poncelet was however able to control the pH in alginate beads by varying the amount of glacial acetic acid added to the oil phase (Poncelet, 2001). Additionally, variations in the spatial distribution of CaCO_3 nanoparticles may cause differences between separate microbeads in the same batch, especially when making small beads which approach the size of the CaCO_3 nanoparticles. The external gelation method of Paques et al. (2012), which uses CaCl_2 nanoparticles dispersed in the oil phase, seems able to avoid the problems of the spatial distribution of particles inside the beads, and the need for an external, acidic trigger to dissociate CaCO_3 . These calcium nanoparticles are created in an oil phase. After emulsification of the polyelectrolyte in the oil phase, this nanocrystals dispersion can be added to the emulsion, and the nanoparticles will diffuse to the interface where they dissolve and cause gelation.

Microstructure is dependent on the balance between attractive and repulsive forces of the building blocks. Fine-stranded protein gels are produced, at low salt concentration and a pH far from the isoelectric point (IEP). Protein gels with a more particulate structure are produced at higher salt concentrations or a pH closer to the IEP (Hongsprabhas, Barbut, & Marangoni, 1999; Remondetto & Subirade, 2003). This microstructure is reflected in the macroscopic

properties of the gels. Particulate gels are usually less elastic and fracture at a small strain and large stress (Ikeda & Morris, 2002; Stading & Hermansson, 1991).

Methods have been developed to create microbeads that could potentially retain oil droplets within their matrix and much is known of different gelation techniques and their relation to gel strength. For microbeads smaller than 25 μ m and containing small particles, the relation between different gelling techniques and the integrity of the microbead after gelling is not well investigated. In this study we compared systems to encapsulate hydrophobic components into microbeads by use of cold set gelation, and investigated the effects of differences in: matrix polymer (alginate and WPI), method of gelation (internal and external gelation), and gelling agent (acid and calcium nanoparticles). The effects of these parameters on gel strength were tested on macroscopic gels, the smoothness of the surface of microbeads without oil was investigated with SEM, and the integrity of microbeads with oil after processing was investigated with CLSM.

2. Materials and Methods

2.1 Materials:

Alginate (Texturas, Barcelona, Spain), Whey protein isolate (WPI) purity 98% (Davisco Foods international, France), CaCO_3 nanoparticles (15-40nm) (purity 97.5%) (SkySpring Nanomaterials, Inc., Houston, USA), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (purity $\geq 99\%$) (GR for analyses, Merck), ethanol absolute (purity $\geq 99.2\%$) (Merck), polyglycerol polyricinoleate (PGPR) 90 kosher (Danisco, Denmark), Medium Chain Triglycerides (MCT) (Miglyol 812 N) (Sasol, Germany), Glucono delta-lactone (GDL) (Fluka Chemika, Switzerland) were all used as received. Solutions were made in demineralized water.

2.2. Methods:

2.2.1. Calcium nanocrystals

Calcium nanocrystals were made according to the method of (Paques, et al., 2012), resulting in a 5% calcium nanocrystal dispersion. In short, 5% (v/v) of a 0.1 molal $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in ethanol solution was emulsified in MCT, containing 6% (w/w) PGPR, with a Sonicator S-250A sonicator (Branson Ultrasonics, USA) for 1 minute. This emulsion was stirred on a magnetic stirrer and heated overnight at 60°C without a cover, allowing the ethanol to evaporate.

2.2.2. Production of the protein aggregates

Protein aggregates were made by making a 10% (w/w) protein solution, which was stirred overnight to allow complete dissolution of the protein. The pH was adjusted to pH 8 with 1M NaOH, and then the solutions were heated at 80°C for 30 minutes while stirring. After heating, the solutions were cooled with tap water and stored at 4°C .

2.2.3. Macro gels

Acid induced protein macro gels were made by the adding GDL to a 10% (w/w) solution of protein aggregates. GDL was added in ratios of 0.1 and 0.2 gram per gram protein (respectively 1 and 2% GDL). CaCO_3 induced protein macro gels were made by adding 30mM of CaCO_3 to the protein aggregate solution, which was then sonicated for 90 seconds (output control level 8, duty cycle 80%). For the CaCO_3 induced alginate macro gels first a 22mM CaCO_3 dispersion was made and sonicated, after which 2.5% (w/w) alginate was added. GDL was then added to both the protein and alginate solution in a GDL/ CaCO_3 molar ration of 1.9/1 to dissociate the CaCO_3 (Paques, Sagis, van Rijn, & van der Linden, 2014). The macro gels were analysed using a rheometer and pH meter (see Sec. 2.2.6 and 2.2.7). Because of the large dimensions of the macro gels, external gelation was inappropriate for making macro gels.

2.2.4. Microbeads without encapsulated oil for SEM analysis

Microbeads without oil droplets were made for SEM analysis. For protein microparticles, a volume ratio of 5% of protein aggregate solution was slowly added to MCT oil containing 2.5% (w/w) PGPR (an oil soluble emulsifier), while mixing with the Ultra-Turrax (T 25, Germany) at 6500 rpm. After full addition, the emulsion was mixed for a further 3 minutes. For alginate microparticles, a volume of 4% of alginate solution was slowly added to MCT oil, containing 4% (w/w) PGPR, while mixing with the Ultra-Turrax at 11500 rpm. After full addition, the emulsion was mixed for a further 90 seconds. For internal gelation gelling agents were added before emulsification in concentrations as mentioned before, and the microparticles were allowed to gel for 20 hours. For external calcium induced gelation, calcium nanocrystals were added to achieve the same concentration of calcium as added in the internally gelled microbeads.

To remove the protein microbeads from the oil phase, the dispersion was centrifuged at 2500 g for 2 hours. To remove the residual oil, the microbeads were washed. The beads were washed by redispersing the pellet in a 2% (w/w) WPI solution, mixed with the Ultra-Turrax at 5000 rpm for 2 minutes, homogenized (Delta instruments, Drachten, The Netherlands) at 100 bars for 2 passes and then centrifuged at 400 g for 40 minutes. This washing step was done twice, the last time redispersing in water instead of a WPI solution. To remove the alginate microbeads from the oil phase, the dispersion was centrifuged at 22000 rpm (48646 g) for 80 minutes. The pellet was redispersed in water and mixed with the Ultra-Turrax at 5000 rpm for 90 seconds, homogenized at 100 bars for 2 passes, and then centrifuged at 660 g for 40 minutes. The pellet was then redispersed in water.

2.2.5. Microbeads with encapsulated oil for CLSM analysis

The inner oil droplets of the protein microbeads were made by adding 5.0% (w/w) MCT to the protein aggregate solution while mixing with the Ultra-Turrax at 10000 rpm for 5 minutes, which was then homogenized at 100 bars for 4 passes. The process then continued as described for the protein microparticles without interior oil droplets (Sec. 2.2.4). The inner oil droplets for the alginate microbeads were made by emulsifying 1% (w/w) WPI and 2.5% (w/w) MCT in water. The mixture was first premixed with the Ultra-Turrax at 8000 rpm for 1 minute and then homogenized at 180 bars for 3 passes. The emulsion was syringe filtered (1.2um) to exclude large droplets and then centrifuged to remove the excess protein. The cream was redispersed in its original volume of water to which 2.5% (w/w) alginate, and in the case of CaCO₃ induced gels also 22mM sonicated CaCO₃, was added. The process then continued as for the alginate microbeads for SEM analysis. In these beads for CLSM analysis, the total amount of matrix polymer to be gelled is lower because of the added volume of oil, which is not present in the beads for SEM analysis. The amount of CaCl₂ nanocrystals was reduced to compensate for this reduction of matrix polymer.

2.2.6. Rheological measurements

Small oscillatory deformation measurements were performed with a Physica MCR 501 Rheometer (Anton Paar, Graz, Austria), with a concentric cylinder measuring geometry (CC17/TI, alginate samples used a sandblasted version of this geometry). Samples were pipetted into the cup immediately after addition of GDL and a thin layer of paraffin oil was applied to prevent evaporation of water. To monitor the gelation process, a frequency of 1 rad/s (0.159 Hz) was applied with a strain of 1% for the protein gels, and 0.002% for the alginate gels. The development of the storage modulus (G') and loss modulus (G'') during 23 hours at 30°C was monitored. To determine the maximum linear strain, a strain sweep was performed at a frequency of 1 rad/s.

2.2.7. pH over time

A volume of 75 mL of alginate or protein aggregate solution was put in a 100 mL Schott bottle, which was put in a waterbath of 30°C. A pH meter with an analogue output was attached to a recorder and the probe was put in the middle of the bottle. After this the gelling agents were mixed into the solution and the pH during gelation was measured for 23 h.

2.2.8. Scanning electron microscopy (SEM)

SEM (Magellan 400, FEI, Eindhoven, the Netherlands) was used to investigate the microstructure of the microbeads. Before viewing, the beads were first adhered to coverslips and critical point dried with carbon dioxide (CPD 030, Baltec, Liechtenstein), and were then sputter coated (Leica EM SCD 500, Leica, Vienna, Austria).

2.2.9. Confocal laser scanning microscopy (CLSM)

CLSM (Leica TCS-SP5, Leica Microsystems B.V., Rijswijk, Netherlands) with an Argon laser was used to image the distribution of oil droplets within the microbead matrix.

214 Dispersions of the protein microbeads were stained with a few drops of 1% Nile Blue
215 (hydrogen sulphate; Merk Millipore, Germany) and analysed immediately after. Nile blue can
216 simultaneously stain MCT oil and whey protein. To visualise the oil, the sample was excited
217 at 488 nm and detected between 505 and 600 nm, to visualise the protein the sample was
218 excited at 633 nm and detected above 650 nm. No adequate stain for alginate could be found.
219 The oil phase was stained with Nile red (0.6 g/L) and the outside water phase was stained
220 with fluorescein isothiocyanate dextran (FITC, MW 40000; Sigma Aldrich, Steinheim,
221 Germany). To visualise Nile red, samples were excited at 488nm and detected above 560 nm.
222 To visualise FITC, the samples were excited at 488 nm and detected between 505 and 600
223 nm.

3. Results and Discussion

3.1 Rheology and pH over time of macroscopic gels

Figure 1 shows the development of the storage modulus and the development of pH over time of the macro gels. The strongest gel is the protein gel gelled with 1% GDL, then the protein gel with 30mM CaCO_3 , then the protein gel with 2% GDL and last the alginate gel with 22 mM CaCO_3 . For acid induced gels the pH of the solution should be close to the IEP to induce gelation. It was found that in the case of whey protein microgels, which are made heating a 4% (w/w) WPI solution at 85°C, the IEP was 4.82 (Schmitt, et al., 2010). In the case of the 1% GDL protein gel, the final pH of 4.8 indeed approaches the pH at the IEP. In the case of the 2% GDL protein gel, the pH goes through the IEP to 4.0. At the IEP, the gel is strongest, but when the pH is lowered further, the strength decreases again. This allows for only a narrow pH range in which to make acid induced, cold set, protein microbeads. When encapsulating an oil-soluble compound, as is done in this study, the pH of the gelled matrix generally has little short term effect on the encapsulated compound (Huang, Frankel, Schwarz, & German, 1996). We note however, that the narrow pH range could pose problems when used to encapsulate other heat sensitive particles such as microorganisms or other living cells, which are very sensitive to the pH.

For CaCO_3 induced gels, only a slightly acidic environment is necessary to dissociate the CaCO_3 and induce gelation. For the protein gel, the pH ends around 6.3, for the alginate gel the pH ends around 5.6. The GDL concentration can be adjusted to have the pH end at any pH below 7.0. This confirms the findings of Poncelet, that the use of an acidic trigger to release CaCO_3 does not necessarily lower the pH to extreme and possibly harmful levels (Poncelet, 2001).

247 The storage modulus for the protein gels is much higher than for the alginate gel. When
248 looking at the strain sweep of the gels (Figure 2) the alginate gel is also much more brittle
249 than any of the protein gels, decreasing in strength around 0.2% strain. The acid induced
250 protein gels show a reduction of strength around 10% strain and the CaCO_3 induced protein
251 gels show a reduction of strength around 50% strain. The CaCO_3 induced protein gels are thus
252 less brittle than the acid induced gels, even though the final gel strength is similar.

253 The strength and elasticity of the beads are very important during processing, especially
254 during the centrifugation and homogenization steps during washing. Centrifugation and
255 homogenization apply harsh forces on the particles, which could mean that they could be
256 made in the oil phase, but not recovered from the oil phase. Our rheological results suggest
257 that the protein beads are more likely to remain intact during processing than the alginate
258 beads, due to the higher strength and elasticity of the protein gels.

3.2 SEM

Figure 3 shows SEM images of the microbeads. Large differences in microstructure are visible. Both the alginate and protein externally gelled microbeads have very smooth surfaces. The surfaces of the acid induced microbeads are quite rough. Further from the IEP the microbeads become more rough. The CaCO_3 induced microbeads have surfaces that are either rough or smooth. This is likely caused by the small dimensions of the microbeads and the high viscosity of both the protein aggregate and the alginate solutions, which caused the CaCO_3 nanoparticles and the GDL to not be homogenously distributed.

Roughness of the surface of the microbeads can be caused by several factors. The concentration of gelling agent might be too high, leading to structures with thicker strands and bigger pores, as is the case for the 2% GDL protein microbeads. It is also possible that the beads shrunk after gelling, causing the surface to shrivel. Additionally, because of inhomogeneous gelation in the bead, as illustrated in Figure 4 (left), the surface of the bead might be weaker and parts of the surface might be peeled off during processing. Last, for internal gelation with calcium, CaCO_3 nanoparticles are used, and some of them might be relatively big compared to the size of the microbead. These particles because of their size need more time to dissolve, which means that the bead may have gelled before the particle is completely dissolved, which may leave holes, and thus weaknesses, in the structure of the bead.

In the external gelation method, the calcium crystals are present in the oil phase and added after emulsification. During gelation the calcium crystals will move to the oil-water interface and the calcium will dissolve there. This method of gelation means that the interface of the droplet gels first and is able to form a 'shell', as illustrated in Figure 4 (right). This shell will

282 increase the strength of the interface of the externally gelled microbeads and prevent
283 shrinking and peeling.

284 The beads with the rough surfaces in the SEM test lack strength, have too much gelling agent,
285 or have an inhomogeneity in the distribution of gelling agent. For the external gelled
286 microbeads the surfaces are smooth, suggesting that a homogeneous and strong gel was made.

3.3 CLSM

CLSM pictures of the microbeads can be found in Figure 5. No fragmented beads can be found in both the alginate and protein externally gelled microbeads. The 1% GDL protein microbeads show some fragmented beads and quite some whole microbeads, the CaCO_3 induced protein microbeads show slightly more fragments, the 2% GDL protein induced microbeads have mostly fragmented. Very few CaCO_3 gelled alginate beads are found. This means that most of the beads have broken, however because of the low density of the alginate gel these fragments have not sedimented during centrifugation, while fragments of protein microbeads have sedimented.

One would reason that the microbeads, which in the rheological tests were the strongest, would have the most resistance to the homogenization and the centrifugation steps during washing, and would thus show the least fragments here. When comparing the results of the 1 and 2% GDL protein microbeads, the results are as hypothesised: the rheological tests show that the 1% GDL is slightly stronger, SEM shows them having a more homogenous and smooth structure, and CLSM shows that more 1% GDL microbeads remain intact throughout the washing process.

Rheology tests showed that CaCO_3 and acid induced protein gels were of similar strength and CLSM showed that microbeads made of these compositions were a mixture of whole beads and fragments. When the calcium source is external, CLSM show only whole beads and no fragments. The externally gelled protein microbeads are therefore much stronger. In SEM they showed a much smoother surface than the internally gelled beads.

Similar as for the protein microbeads, the internal and external gelled alginate microbeads show large differences in strength, despite the equal amount of calcium and alginate in the systems. According to the rheological tests, the internally gelled alginate gel was the weakest.

311 The CLSM results show that the internally gelled alginate microbeads are indeed some of the
312 weakest, however the externally gelled alginate microbeads are some of the strongest,
313 stronger than the internally gelled protein microbeads. This means that the way in which the
314 calcium is introduced into the system, and thus the smoothness of the beads that results, is
315 very important to the integrity of microbeads of this scale. This supports the theory that the
316 calcium diffusing from outside into the microbead causes the formation of a strong, smooth
317 shell, which protects the microbeads from destruction during processing.

4. Conclusions

In this study the importance of matrix polymer, method of gelation, and gelling agent on the strength and integrity of microbeads, smaller than 25 μm , containing oil droplets was studied. Both alginate and protein externally gelled microbeads have smooth surfaces and are sufficiently strong to survive centrifugation and homogenization steps in the production process. The CaCO_3 , internally gelled microbeads, containing the same amount of calcium, fragmented during production. This means that the method in which the calcium is introduced into the beads is important.

Externally gelled alginate beads were stronger than acid and CaCO_3 gelled protein microbeads, even though in macroscopic gels the CaCO_3 gelled alginate gel was much weaker and more brittle than the CaCO_3 gelled protein gel. This means that the gelling mechanisms can be more important than the type of matrix polymer.

Acid induced gelation of the protein microbeads gives slightly rough microbeads and a strength which allows only half of the microbeads to remain intact throughout the production process. The pH range in which the strength is optimal is quite narrow and the current beads cannot be significantly improved further by further optimising the GDL concentration. Acid induced gelation is therefore not an adequate alternative of gelation for these types of microbeads.

This investigation shows that the technique of external gelation is more important to microbead integrity than the type of matrix polymer, and results in microbeads with mechanical properties superior to those produced with internal and acid induced gelation.

339 Acknowledgement

340 The research presented in this paper was financially supported by the Graduate School
341 VLAG, of Wageningen University.

References:

- Allan-Wojtas, P., Truelstrup Hansen, L., & Paulson, A. T. (2008). Microstructural studies of probiotic bacteria-loaded alginate microcapsules using standard electron microscopy techniques and anhydrous fixation. *LWT - Food Science and Technology*, 41(1), 101-108.
- Annan, N. T., Borza, A. D., & Hansen, L. T. (2008). Encapsulation in alginate-coated gelatin microspheres improves survival of the probiotic *Bifidobacterium adolescentis* 15703T during exposure to simulated gastro-intestinal conditions. *Food Research International*, 41(2), 184-193.
- Beaulieu, L., Savoie, L., Paquin, P., & Subirade, M. (2002). Elaboration and Characterization of Whey Protein Beads by an Emulsification/Cold Gelation Process: Application for the Protection of Retinol. *Biomacromolecules*, 3(2), 239-248.
- Bryant, C. M., & McClements, D. J. (1998). Molecular basis of protein functionality with special consideration of cold-set gels derived from heat-denatured whey. *Trends in Food Science & Technology*, 9(4), 143-151.
- Chan, L. W., Lee, H. Y., & Heng, P. W. S. (2006). Mechanisms of external and internal gelation and their impact on the functions of alginate as a coat and delivery system. *Carbohydrate Polymers*, 63(2), 176-187.
- Draget, K. I., Skjåk Bræk, G., & Smidsrød, O. (1994). Alginic acid gels: the effect of alginate chemical composition and molecular weight. *Carbohydrate Polymers*, 25(1), 31-38.
- Gombotz, W. R., & Wee, S. F. (2012). Protein release from alginate matrices. *Advanced Drug Delivery Reviews*, 64, Supplement, 194-205.
- Gunasekaran, S., Ko, S., & Xiao, L. (2007). Use of whey proteins for encapsulation and controlled delivery applications. *Journal of Food Engineering*, 83(1), 31-40.

366 Hongsprabhas, P., Barbut, S., & Marangoni, A. G. (1999). The Structure of Cold-Set Whey
 367 Protein Isolate Gels Prepared With Ca⁺⁺. *LWT - Food Science and Technology*, 32(4),
 368 196-202.

369 Huang, S.-W., Frankel, E. N., Schwarz, K., & German, J. B. (1996). Effect of pH on
 370 antioxidant activity of α -tocopherol and trolox in oil-in-water emulsions. *Journal of*
 371 *Agricultural and Food Chemistry*, 44(9), 2496-2502.

372 Ikeda, S., & Morris, V. J. (2002). Fine-Stranded and Particulate Aggregates of Heat-
 373 Denatured Whey Proteins Visualized by Atomic Force Microscopy.
 374 *Biomacromolecules*, 3(2), 382-389.

375 Ince Coskun, A. E., Saglam, D., Venema, P., van der Linden, E., & Scholten, E. (2015).
 376 Preparation, structure and stability of sodium caseinate and gelatin micro-particles.
 377 *Food Hydrocolloids*(0).

378 Kaushik, V., & Roos, Y. H. (2007). Limonene encapsulation in freeze-drying of gum Arabic–
 379 sucrose–gelatin systems. *LWT - Food Science and Technology*, 40(8), 1381-1391.

380 Kuhn, P. R., & Foegeding, E. A. (1991). Mineral salt effects on whey protein gelation.
 381 *Journal of Agricultural and Food Chemistry*, 39(6), 1013-1016.

382 Li, Y., Hu, M., Du, Y., Xiao, H., & McClements, D. J. (2011). Control of lipase digestibility
 383 of emulsified lipids by encapsulation within calcium alginate beads. *Food*
 384 *Hydrocolloids*, 25(1), 122-130.

385 Liu, X. C., Yu, W. Y., Zhang, Y., Xue, W. M., Yu, W. T., Xiong, Y., Ma, X. Y., Chen, Y., &
 386 Yuan, Q. (2002). Characterization of structure and diffusion behaviour of Ca-alginate
 387 beads prepared with external or internal calcium sources. *Journal of*
 388 *Microencapsulation*, 19(6), 775-782.

389 Lupo, B., Maestro, A., Porras, M., Gutiérrez, J. M., & González, C. (2014). Preparation of
 390 alginate microspheres by emulsification/internal gelation to encapsulate cocoa
 391 polyphenols. *Food Hydrocolloids*, 38(0), 56-65.

392 McClements, D. J. (2012). Advances in fabrication of emulsions with enhanced functionality
 393 using structural design principles. *Current Opinion in Colloid & Interface Science*,
 394 17(5), 235-245.

395 Paques, J. P., Sagis, L. M. C., van Rijn, C. J. M., & van der Linden, E. (2014). Nanospheres
 396 of alginate prepared through w/o emulsification and internal gelation with
 397 nanoparticles of CaCO₃. *Food Hydrocolloids*, 40(0), 182-188.

398 Paques, J. P., van der Linden, E., van Rijn, C. J. M., & Sagis, L. M. C. (2012). Alginate
 399 submicron beads prepared through w/o emulsification and gelation with CaCl₂
 400 nanoparticles. *Food Hydrocolloids*, 31(2), 428-434.

401 Paques, J. P., van der Linden, E., van Rijn, C. J. M., & Sagis, L. M. C. (2014). Preparation
 402 methods of alginate nanoparticles. *Advances in Colloid and Interface Science*, 209(0),
 403 163-171.

404 Poncelet, D. (2001). Production of Alginate Beads by Emulsification/Internal Gelation.
 405 *Annals of the New York Academy of Sciences*, 944(1), 74-82.

406 Remondetto, G. E., & Subirade, M. (2003). Molecular mechanisms of Fe²⁺-induced β-
 407 lactoglobulin cold gelation. *Biopolymers*, 69(4), 461-469.

408 Sağlam, D., Venema, P., de Vries, R., Sagis, L. M. C., & van der Linden, E. (2011).
 409 Preparation of high protein micro-particles using two-step emulsification. *Food*
 410 *Hydrocolloids*, 25(5), 1139-1148.

411 Schmitt, C., Moitzi, C., Bovay, C., Rouvet, M., Bovetto, L., Donato, L., Leser, M. E.,
 412 Schurtenberger, P., & Stradner, A. (2010). Internal structure and colloidal behaviour

of covalent whey protein microgels obtained by heat treatment. *Soft Matter*, 6(19),
4876-4884.

Smidsrød, O., & Skjåk-Bræk, G. (1990). Alginate as immobilization matrix for cells. *Trends
in Biotechnology*, 8(0), 71-78.

Stading, M., & Hermansson, A.-M. (1991). Large deformation properties of β -lactoglobulin
gel structures. *Food Hydrocolloids*, 5(4), 339-352.

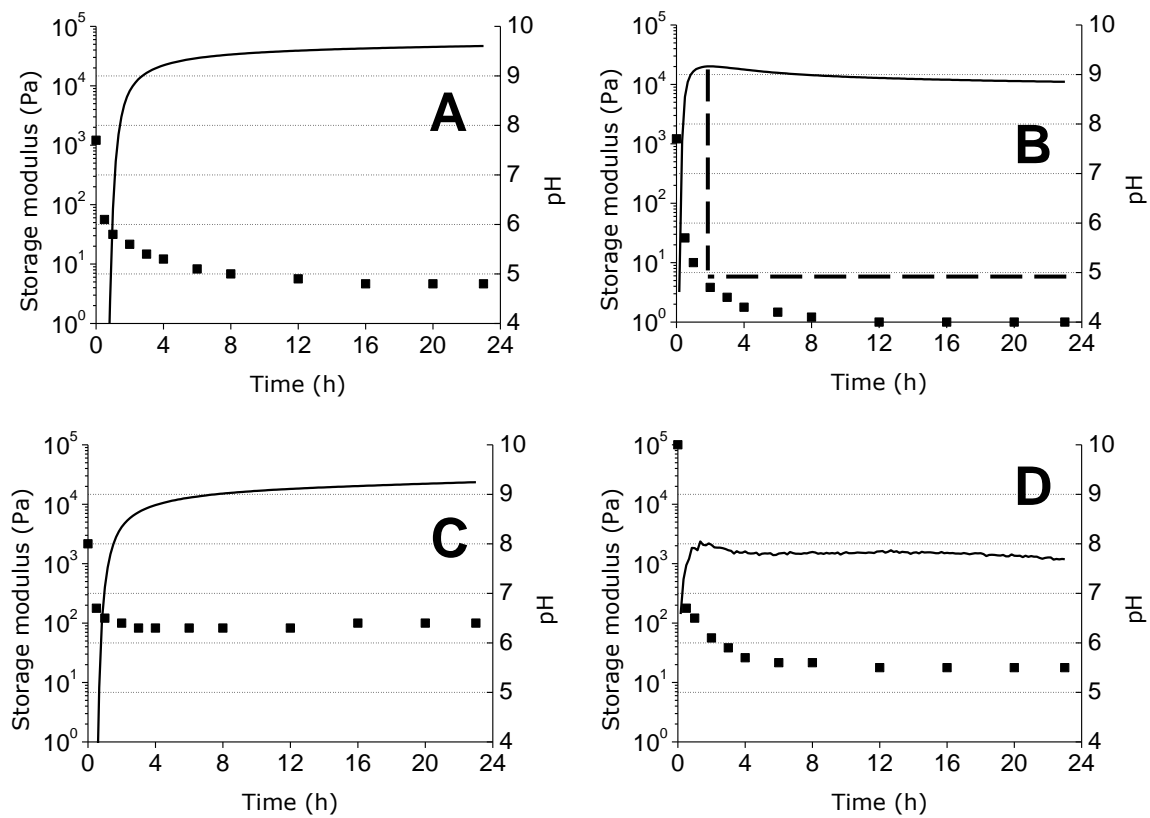
Tyle, P. (1993). Tactile Pattern Recognition Effect of size, shape and hardness of particles in
suspension on oral texture and palatability. *Acta Psychologica*, 84(1), 111-118.

Walther, B., Walkenström, P., Hermansson, A.-M., Fischer, P., & Windhab, E. J. (2002).
Flow processing and gel formation—a promising combination for the design of the
shape of gelatin drops. *Food Hydrocolloids*, 16(6), 633-643.

Yoo, S.-H., Song, Y.-B., Chang, P.-S., & Lee, H. G. (2006). Microencapsulation of α -
tocopherol using sodium alginate and its controlled release properties. *International
Journal of Biological Macromolecules*, 38(1), 25-30.

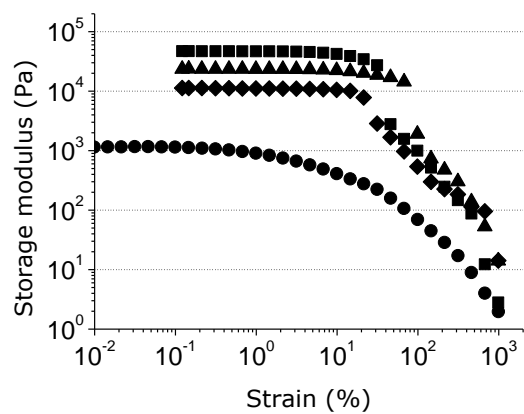
428 Figures:

429 Figure 1:



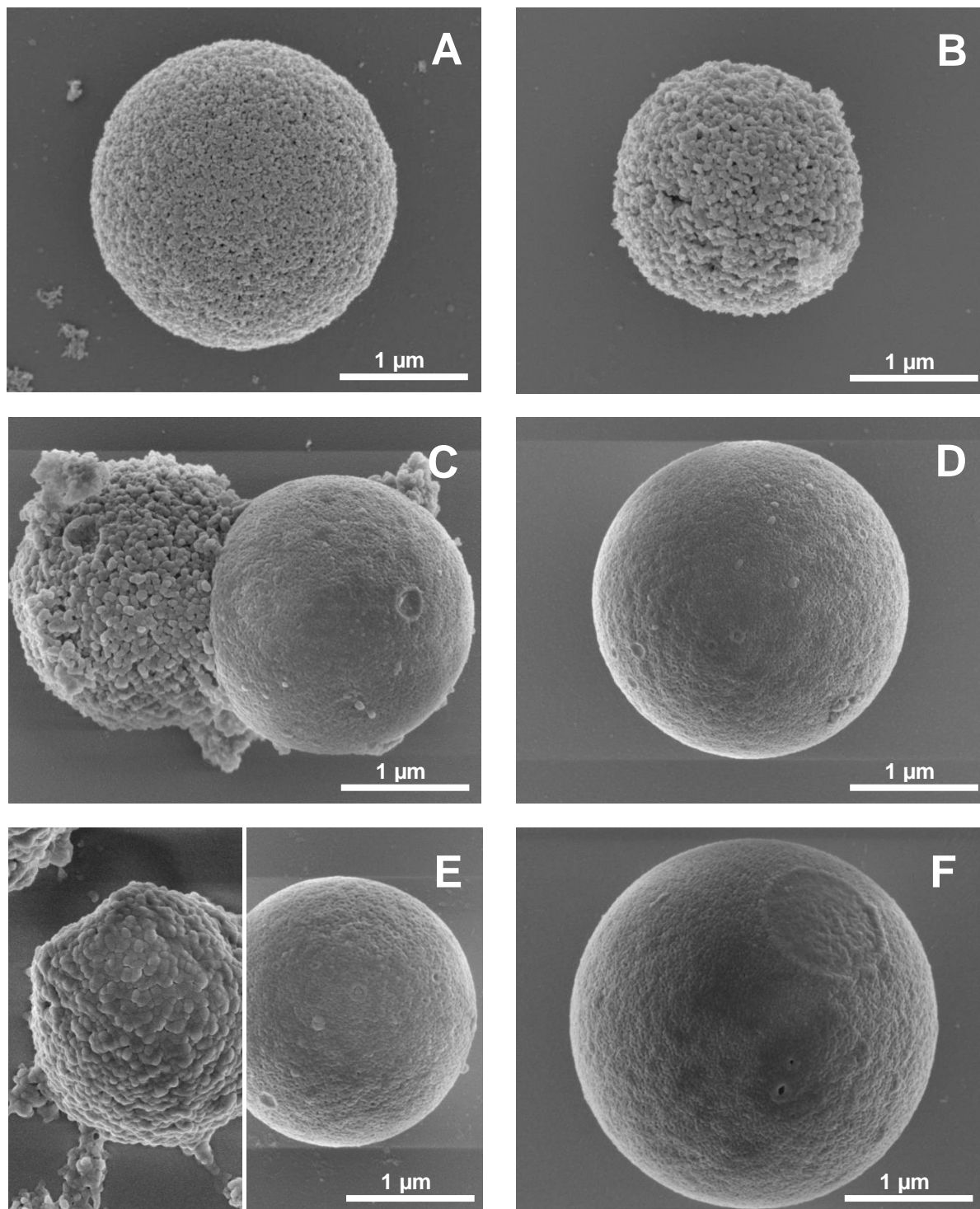
430

431 Figure 2:



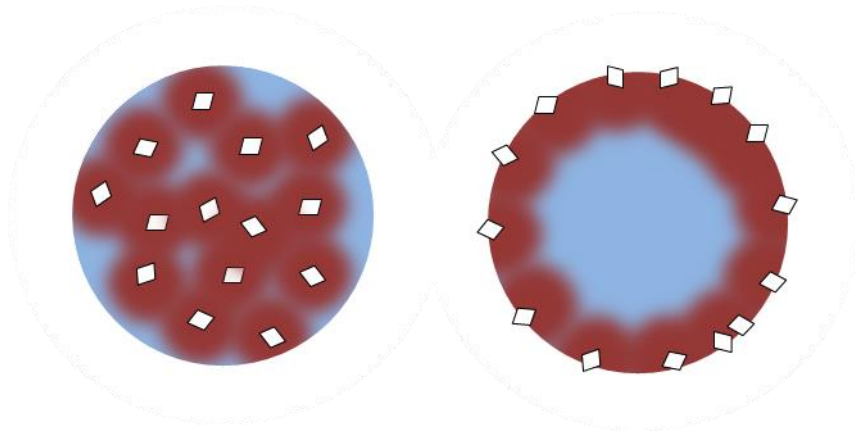
432

433 Figure 3:



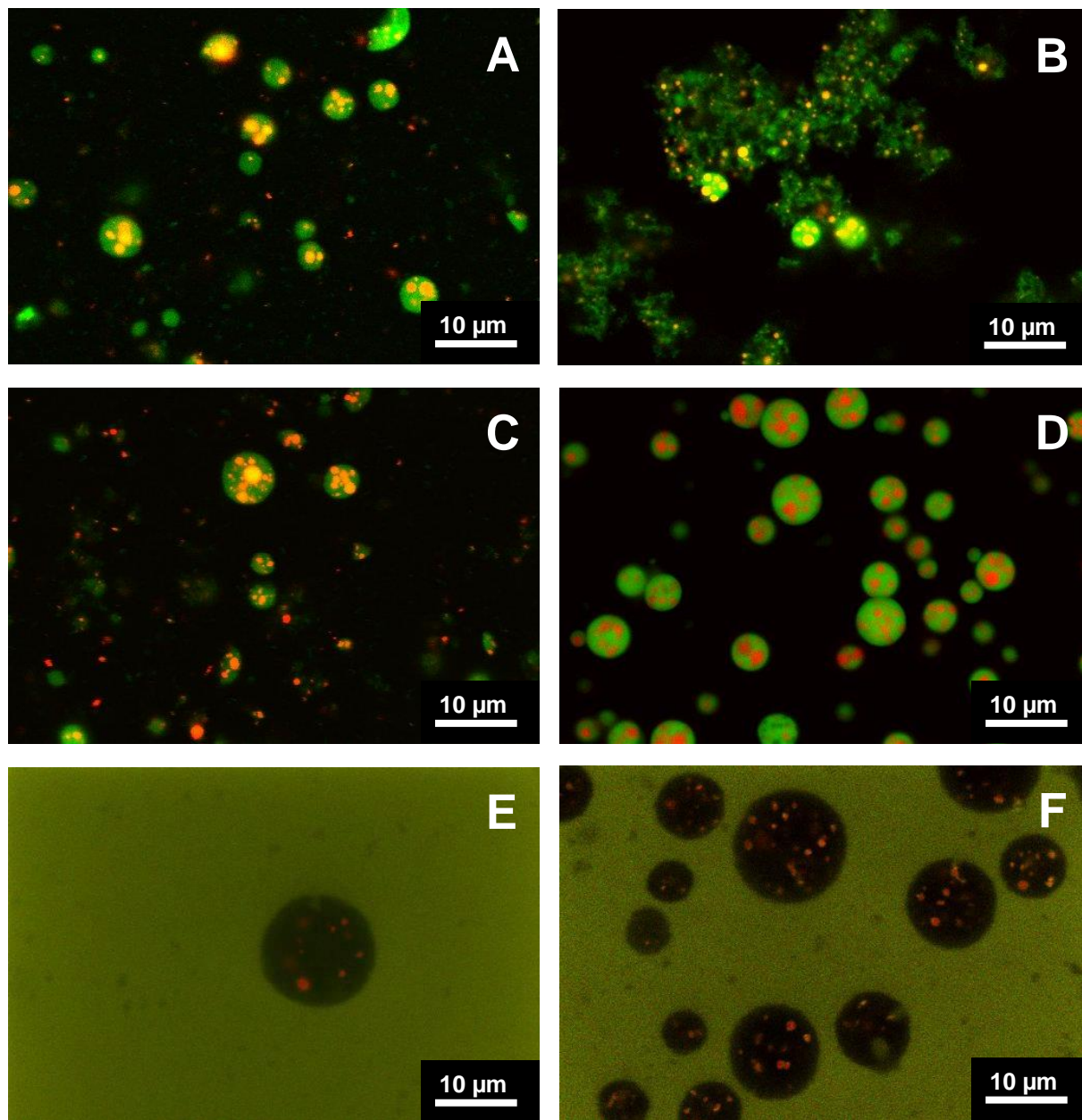
434

435 Figure 4:



436

437 Figure 5:



438