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Soybean oleosome-based oleogels *via* polymer-bridging based structuring. Mechanical properties at large deformations[☆]

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Abstract – Oleosomes have emerged in the last decade as a multipurpose oil-in-water emulsion suitable for engineering new lipids materials. The need to increase alternative and sustainable methods to modulate the rheological properties of emulsions has been the leading research interest in the oleogelation field. Soybean oleosomes are evaluated as building blocks for creating oleogels in combination with sodium alginate or ι-carrageenan as the structuring elements. Polymer bridging provides a route to produce compact soft, malleable gels by exploiting attractive electrostatic interactions between negatively charged polysaccharides and oleosome surfaces. We investigated the viscoelastic properties of concentrated polymer bridged gels by oscillatory rheological measurements. The rheological characteristics are governed predominantly by the type of polysaccharide and by the ratio between polysaccharide and oleosome content. One yielding step at low strains indicates the breaking of polysaccharide bridging bonds and was visible in all samples. A two-step yielding process, where the second step corresponds to the cage-breaking process, was present at polysaccharide/oleosome ratios where optimum bridging occurs for alginate and carrageenan, 0.005 g/g, 0.01 g/g, respectively. Nonetheless, the bumps corresponding to the second yielding point were more prominent in alginate gels than in carrageenan gels due to the greater bridging ability of alginate. Identifying these rheological hallmarks could provide new ideas towards the improved design of plant-based fatty food products, *e.g.*, cream cheeses and vegan sausages surrogates.

Keywords: lipid droplets / oleogel / soybean / oil structuring / polysaccharides / electrostatic interactions

Résumé – **Des oléogels à base d'oléosomes de soja *via* une structuration basée sur le pontage de polymères. Propriétés mécaniques au cours de grandes déformations.** Les oléosomes sont apparus au cours de la dernière décennie comme une émulsion huile-dans-l'eau polyvalente, adaptée à l'ingénierie de nouveaux matériaux lipidiques. La nécessité d'augmenter les méthodes alternatives et durables pour moduler les propriétés rhéologiques des émulsions a été le principal intérêt de la recherche dans le domaine de l'oléogélation. Les oléosomes de soja sont évalués en tant qu'élément constitutifs pour créer des oléogels en combinaison avec de l'alginate de sodium ou de la ι-carraghénine comme éléments structurants. Le pontage des polymères permet de produire des gels compacts, mous et malléables en exploitant les interactions électrostatiques attractives entre les polysaccharides chargés négativement et les surfaces des oléosomes. Nous avons étudié les propriétés viscoélastiques de gels concentrés de polymères pontés, par des mesures rhéologiques oscillatoires. Les caractéristiques rhéologiques sont régies principalement par le type de polysaccharide et par le rapport entre la teneur en polysaccharides et en oléosomes. Une étape de déformation à une faible contrainte, correspondant à la rupture des liaisons avec le polysaccharide, est observée pour des ratios polysaccharide/oléosome (0,005 g/g d'alginate, 0,01 g/g de carraghénane) où l'efficacité du pontage n'est pas optimale. Un processus de déformation en deux étapes, où la deuxième étape correspond au processus de rupture de structures de type cages, s'exerce pour des ratios polysaccharides/oléosomes où le pontage est optimal pour l'alginate et la carraghénine, 0,005 g/g, 0,01 g/g, respectivement. Néanmoins, les épaulements correspondant à la seconde limite d'élasticité étaient plus importantes dans les gels d'alginate que dans les gels de carraghénane en raison de la plus grande capacité de

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pontage de l'alginate. L'identification de ces caractéristiques rhéologiques pourrait fournir de nouvelles idées pour améliorer la conception de produits alimentaires gras à base de plantes, par exemple, des fromages à la crème et des substituts de saucisses végétaliennes.

Mots clés : gouttelettes lipidiques / oléogel / soja / structuration de l'huile / polysaccharides / interactions électrostatiques

1 Introduction

Polymer bridging has been proposed as a structuring method for natural oil-in-water emulsions, so-called oleosomes. Polymer bridging requires that polymeric chains adsorb simultaneously on multiple oil droplets to create a percolated gel network (Yu and Somasundaran, 1996). Previous findings showed that bridging flocculation, induced by negatively charged polysaccharides, formed a heterogeneous and interconnected network of oleosome droplets (Zambrano and Vilgis, 2022). Sodium alginate proved to be a very effective polysaccharide for inducing bridging flocculation of oleosome droplets where its optimum bridging coverage occurs at 0.005 g/g. In comparison, the optimum bridging coverage of ι-carrageenan occurs at 0.01 g/g (Zambrano and Vilgis, 2023); this difference in bridging ability between alginate and carrageenan results from structural conformations. Alginate polymer chains are organized in a co-block arrangement of charged and uncharged units of mannuronic acid and guluronic acids groups (Ahn *et al.*, 2019), thus enabling flexible polymeric chains to interconnect multiple oleosome droplets. The charges in the carrageenan chain are organized uniformly along its chain (Piculell, 2006), which makes it more efficient for complete surface coating of the oleosome surface. However, polymer chains are less available to interconnect multiple oleosome droplets into a percolated network.

The structural differences between both polysaccharides will also influence the microstructure of the gel network. Polymer bridging gels induced by alginate presented large and interconnected clusters, resulting in a gel network with large pores where water can easily migrate. On the other hand, ι-carrageenan-induced gels presented smaller and less interconnected clusters with smaller pores. Thus, water is less available to be separated, so the bridging efficiency decreases. Therefore, the gel microstructure will determine the extent of water separation from the droplet network. Centrifugation can quickly accelerate the network contraction and water separation process. As a result of bridging flocculation, the droplet network contracts upwards due to the relatively higher density of the oil droplet network than the water phase in which they are dispersed. Centrifugation allows the flocculated emulsions to form a continuous mass in which the water is easily removed, resulting in a low-moisture soft, malleable gel. This separation process is analogous to other processes in the food industry, such as the production of cream cheese in which the fat-containing curd is separated from a liquid phase using a cream-type separator or a quark-type separator by application of centrifugal force.

Therefore, the physical properties of the oleogels can be systematically altered by changing process conditions, or changing preparation conditions, such as oleosome content,

polymer type and polymer/oleosome ratio. This interplay of conditions can result in soft gels with different shapes or volumes, which could be efficiently designed to produce food products with different textural attributes. The current study builds on the previous work on polymer bridging emulsions induced by sodium alginate and ι-carrageenan (Zambrano and Vilgis, 2023). Here, it was also shown that differences in gel microstructure would influence the viscoelastic properties of the resulting gel material. Bridging flocculated emulsions at the optimum bridging ratio presented fracture-like behavior at deformations larger than the linear viscoelastic range ($\sim 3\%$). This is due to the strong and irreversibly bonds in the microstructural elements formed at the optimum bridging ratio. In addition, polymer-bridging gels presented a characteristic overshoot in the modulus at larger deformations ($\sim 200\%$), which needs to be further, elucidated. Therefore, this study investigates the rheological properties of compacted oleosome-based gels formed by polymer bridging. For this purpose, the effect of polysaccharide/oleosome ratio between sodium alginate and ι-carrageenan are compared to analyze the effect on the mechanical properties at large deformation as determined by oscillatory rheological measurements.

2 Materials and methods

2.1 Materials

Commercially available soybeans were purchased from Rapunzel Naturkost GmbH (Legau, Germany) and were used to extract oleosomes. Sodium alginate (TICA-algin 400 Powder) was provided by TIC Gums, Inc. (Belkamp, Md., USA). Iota carrageenan was provided by Carl Roth (Karlsruhe, Germany). Distilled water was used for the preparation of all solutions.

2.2 Sample preparation

Soybean oleosomes extraction is based on the method by Waschatko *et al.* (2012a). Soybeans were soaked in distilled water at 4 °C for at least 20 h. Then, water was added to obtain a 10% soybean-to-water ratio, ground in a Vorwerk Thermomix TM31 at 10 200 rpm for 90 s. The resulting slurry was filtered through two layers of Kimtech wipes 21×11 cm (Kimberly Clark) to obtain raw milk. 25 wt% sucrose was added to the raw milk, and the pH was adjusted to 11.0 with $1 \, N \cdot NaOH$ (VWR Chemicals) solution. The solution was filled into 50 mL centrifuge tubes (Roth) and centrifuged at $15\,000 \times g$ at 4 °C for at least 5 h. The resulting floating fractions (cream layer) were collected with a small spoon and resuspended in a new centrifuge tube in 20 wt% Sucrose in distilled water (pH 11.00). A new washing and centrifugation step ($15\,000 \times g$, 4 °C, 5 h) was repeated, and the resulting

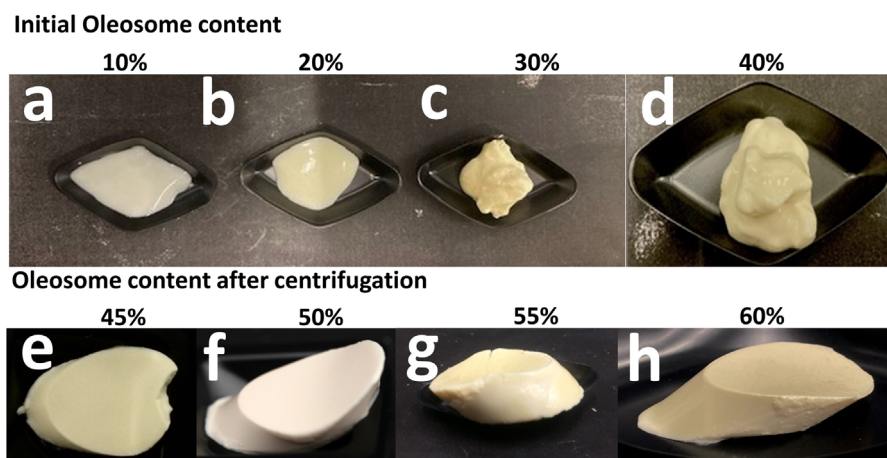**Initial Oleosome content**

Fig. 1. Visual appearance of polymer bridged gels of alginate and carrageenan–oleosome mixtures. Samples from the top correspond to samples before centrifugation, and samples from the bottom correspond to the same samples after centrifugation. a, c, e, g, corresponded to carrageenan, and b, f, d, h corresponded to alginate.

oleosome cream was collected in a centrifuge tube. This method removes unspecific bounded soybean storage proteins (glycinin and β-conglycinin) and other allergenic proteins (Gly m BD 30 K) from the surface of the oleosomes (Waschatko *et al.*, 2012b). The extracted oleosome cream had 50–40% water content as measured by a halogen dryer. The dry weight of the cream is assumed as the oleosome content. Since the precise composition of oleosomes is unknown, theoretical values of the three major components of soybean oleosome were used according to Huang's (1992). This author calculated theoretical values of oleosome components according to the oleosome diameter size. As determined by laser diffraction analysis (Zambrano and Vilgis, 2022), soybean oleosomes referred to the current extraction method resulted in a particle size of $0.350\,\mu m$. This model calculates the amounts of approximately 3.3% (w/w) phospholipids, 5.2% (w/w) protein, and 91.5% (w/w) triglycerides for an oleosome with a $0.350\,\mu m$ diameter.

2.2.1 Preparation of bridging flocculated gels

The initial pH of the oleosome emulsion was adjusted to 7.0 with 1 NaOH. Dried sodium alginate and carrageenan samples were added directly to the oleosome emulsion at different concentrations to form different mass ratios (g polysaccharide/g oleosome). Polysaccharides–oleosome mixtures were stirred for a minimum of 20 h to ensure proper hydration. Afterward, polysaccharides–oleosome mixtures were slowly adjusted to pH 4.0 *via* dropwise addition of HCl with varying concentrations (1.0, 0.5, 0.1, 0.01 N) at a constant stirring speed of 450 rpm. Bridging flocculated polysaccharide–oleosome mixtures were centrifuged $(5000 \times g, 4\,^{\circ}C, 20\,min)$ in 50 mL centrifuges tubes. The supernatant was decanted, and the compacted gels were then carefully collected.

2.3 Rheology measurements

Oscillatory rheology was performed on compacted gels in order to investigate their resulting viscoelastic properties.

Measurements were performed with a Bohlin Instruments Gemini 200 rheometer (Malvern Panalytical Ltd., Malvern, UK) equipped with 25-mm parallel plate geometry. The gap size was adjusted stepwise to the thickness of the gels until the load in the normal force was detected, leading to gap sizes between $1000–1500\,\mu m$. Wall slip effect was discarded by measuring at different gaps while keeping other measuring conditions constant. After loading, a 5-min waiting period at $25\,^{\circ}C$ was used to allow the sample's structure to relax before the measuring process began. Oscillatory amplitude sweeps were performed by increasing the strain logarithmically from 0.01 to 1000% at 1 Hz. All measurements were performed in triplicate from different batches of gels and all samples were prepared individually.

2.4 Microstructural observation

The microstructure of the concentrated gels was observed by scanning electron microscopy (LEO Gemini 1530) under a suitable accelerating voltage. Upon centrifugation, concentrated gels were plunged into liquid nitrogen before being placed in a freeze dryer (Christ Alpha 1-2 LD plus). Samples were left to dry overnight, and afterward, the samples were stored in a sealed glass container at room temperature. Subsequently, the dried samples were carefully placed on standard aluminum stubs (Plano GmbH; Wetzlar, Germany), attached with double-sided carbon tapes, and transferred to the microscope under vacuum.

3 Results and discussions

3.1 Visual appearance of polymer bridged gels

The visual appearance of polymer bridging gels before and after centrifugation containing increasing concentrations of oleosome and alginate and ι-carrageenan are shown in Figure 1. As earlier observed (Zambrano and Vilgis, 2022), self-standing compact gels were obtained upon centrifugation in which the resulting concentration will increase as the initial oleosome content increases. As previously discussed (Zambrano and
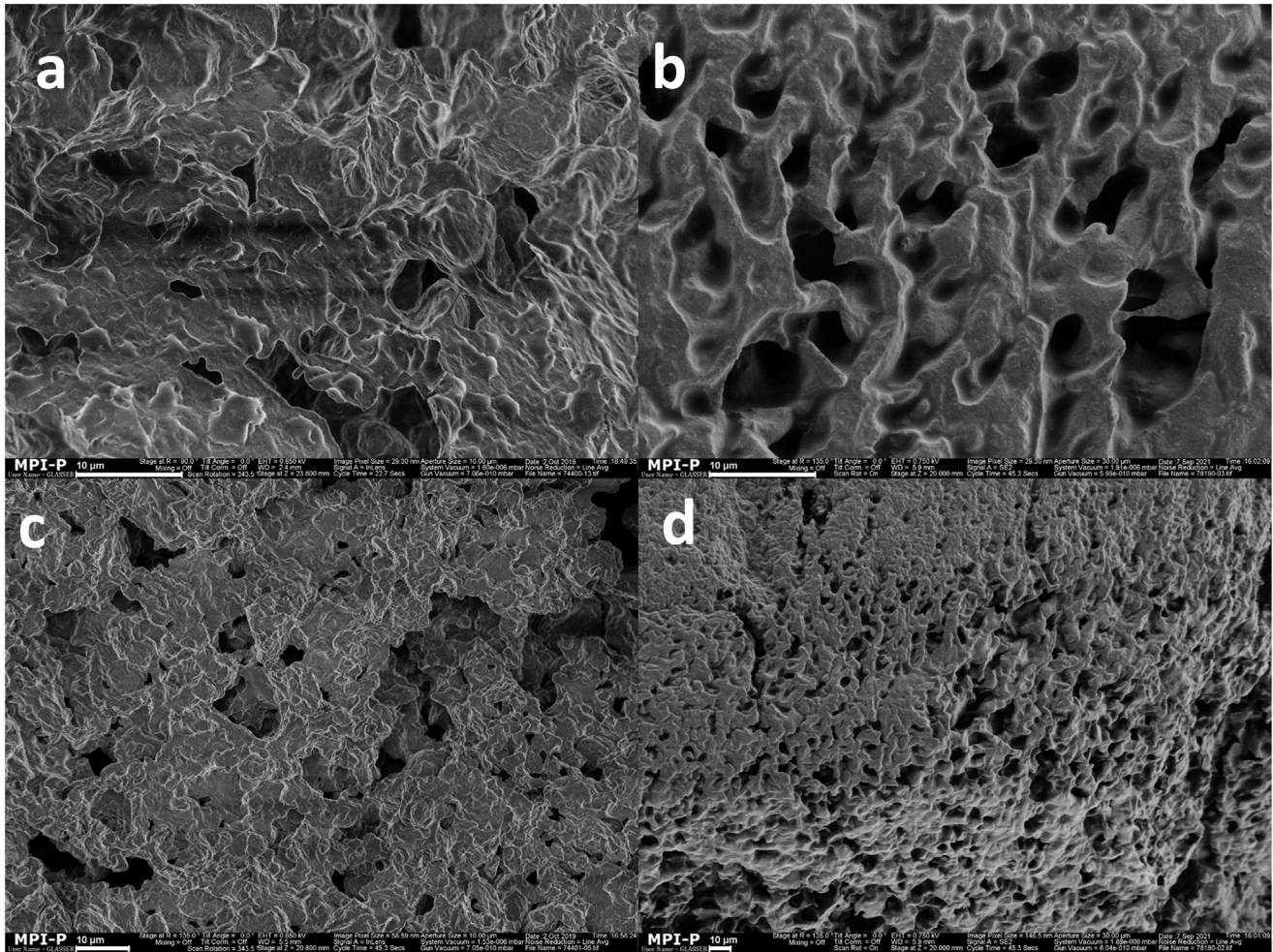
Fig. 2. SEM images of polymer-bridged gels upon densification 45 wt% oleosome fixed at 0.005 g/g. (a–c) carrageenan and (b–d) alginate. Scale bars: 10 μm.

Vilgis, 2022), droplet networks flocculated by the polymer bridging mechanism will contract upwards due to the density difference between the oil and the continuous water phase. By accelerating this separation, the droplet network can be easily concentrated, resulting in a compact gel in which the viscoelastic properties can be expanded.

3.2 Microstructure of polymer bridged gels

Figure 2 shows SEM images depicting the microstructure of the compacted gels by alginate and carrageenan. Carrageenan gels (Figs. 2a and 2c) present a rougher microstructure, while alginate presents a smoother microstructure (Figs. 2b and 2d). A rougher microstructure is likely due to loops of chains from carrageenan, which may be dangling, whereas alginate, due to more efficient use of its polymer chains, is less likely to present dangling loops around its microstructure.

3.3 Rheological characterization of polymer-bridged gels

The results of oscillatory strain sweeps of the polymer-bridged gels upon densification are shown in Figure 3. Overall

G' values (10^3–10^4 Pa) in the linear viscoelastic region (LVR) were higher than those previously reported (Zambrano and Vilgis, 2022) of non-centrifuged bridging flocculated oleosomes. This shows that the high density of the oleosome clusters upon centrifugation determines the bulk modulus in the LVR. The effect of cluster density is more clearly observed in 0.005 g/g alginate (Fig. 3b), where G' values in the LVR are the same order of magnitude for all oleosome concentrations. At this ratio, alginate presents its optimum bridging ratio in which alginate chains effectively bridge all oleosome droplets. Thus, dense interconnected clusters determine the high density at the different oleosome concentrations.

Similarly, for 0.01 g/g carrageenan (Fig. 3c), the moduli in the LVR do not vary significantly, at least for oleosome concentrations > 10 wt%. As previously reported (Zambrano and Vilgis, 2022), the optimum bridging ratio in carrageenan occurs at 0.01 g/g where, as observed for 0.005 g/alginate, dense clusters determine the bulk modulus in the LVR. On the other hand, the moduli values in the LVR differed across the various oleosome contents at ratios further from the optimal bridging ratio for alginate and carrageenan (0.01 g/g and 0.005 g/g, respectively) (Figs. 3a and 3d). As the ratio between polymer and droplets are farther from the optimum ratio for
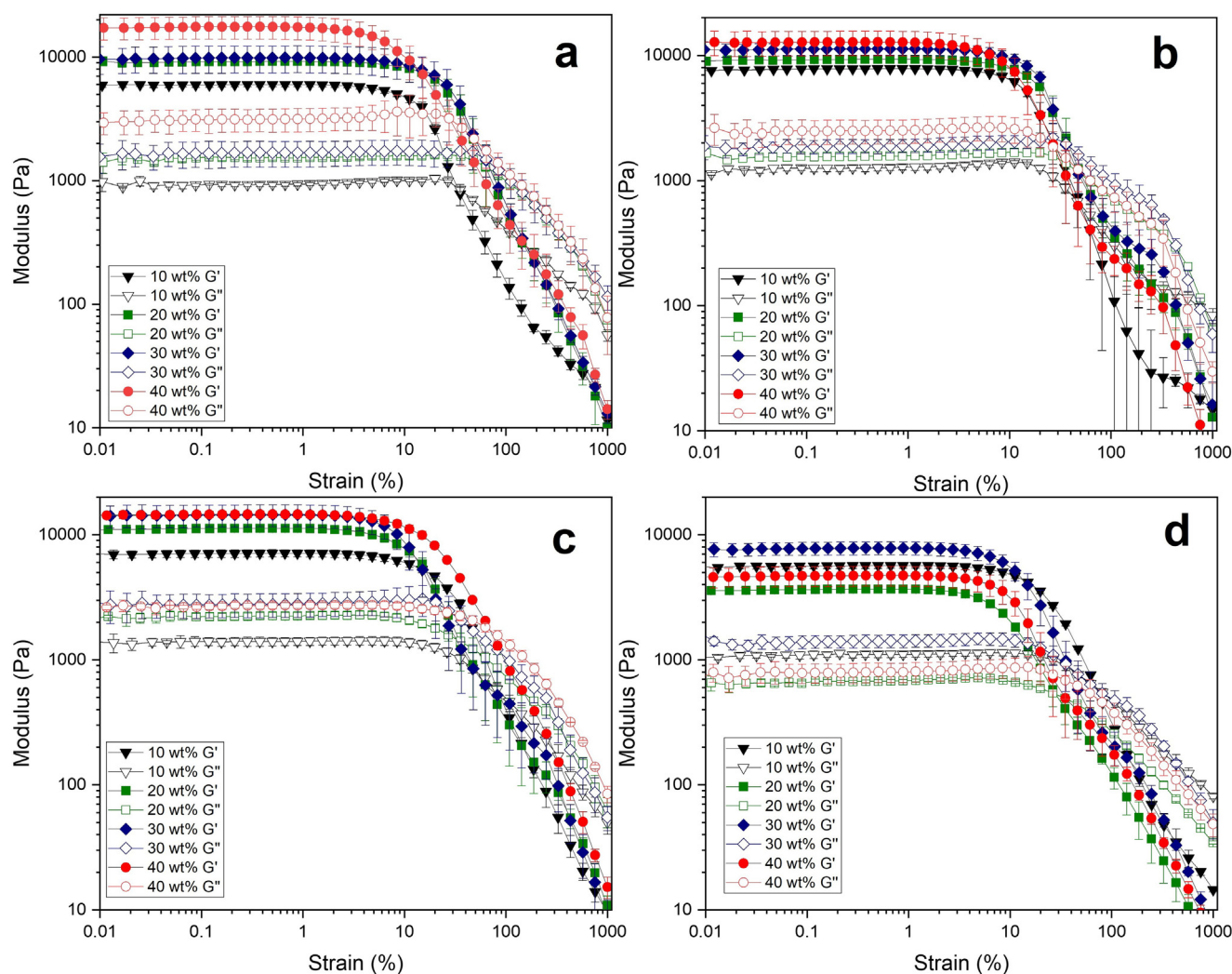
Fig. 3. Oscillatory strain sweeps of polymer-bridged gels after centrifugation at different oleosome contents and fixed at two polysaccharide/oleosome ratios. (a) 0.005 g carrageenan/g oleosome; (b) 0.005 g alginate/g oleosome; (c) 0.01 g carrageenan/g oleosome; (d) 0.01 g alginate/g oleosome.

efficient bridging flocculation to occur, polymer bridges between droplets start to disappear (Zambrano and Vilgis, 2022). As a result, clusters upon centrifugation will be less interconnected and thus less dense at these ratios (Zambrano and Vilgis, 2022), causing bulk density not to be relevant anymore for the modulus in the LVR.

3.3.1 Focus on the effects of large deformations

The most remarkable difference among the samples occurs at strains around $\gamma = 300\%$, with a small hump in G' and G'' appearing. As previously observed (Zambrano and Vilgis, 2022), a strain overshoot is visible at higher strains, confirming a two-step yielding behavior. In order to present this yielding behavior more clearly, from the same oscillatory measurements, the stress (σ) is plotted against strain (γ) in Figure 4.

First, it is observed that stress response is linearly dependent on strain, indicating stress behaves elastically at strains up to 20%, which is followed by a first yielding point (γ_1) that seems to be independent of oleosome content (Fig. 5).

The first yielding point (γ_1) is likely to indicate the confinement by density plus polysaccharide bridging breakage between the oil droplets. As the polysaccharide concentration is fixed at two ratios, 0.005 g/g and 0.01 g/g, the bridging bonds can be assumed the same for the different oleosome concentrations. This is supported in Figure 5, where the values of the first yielding strain γ_1 among the samples do not significantly change for the different oleosome concentrations. Only small variations, however, can be depicted for carrageenan (Figs. 4a and 4c). At 0.005 g/g and 0.01 g/g carrageenan, γ_1 slightly varies with oleosome content, albeit not significantly to the point of showing any dependence with oleosome content, as observed in Figure 5. These slight variations can be explained by the weaker bridging ability of carrageenan, which fails to form tighter bridging bonds in contrast to alginate (see Fig. 6). This contrasts with 0.005 g/g alginate (Fig. 4b), where γ_1 does not change with oleosome content. Similar as carrageenan, for 0.01 g/g alginate (Fig. 4d), γ_1 slightly varies with oleosome content due to the decreased bridging ability of alginate at this ratio. Consequently, the first
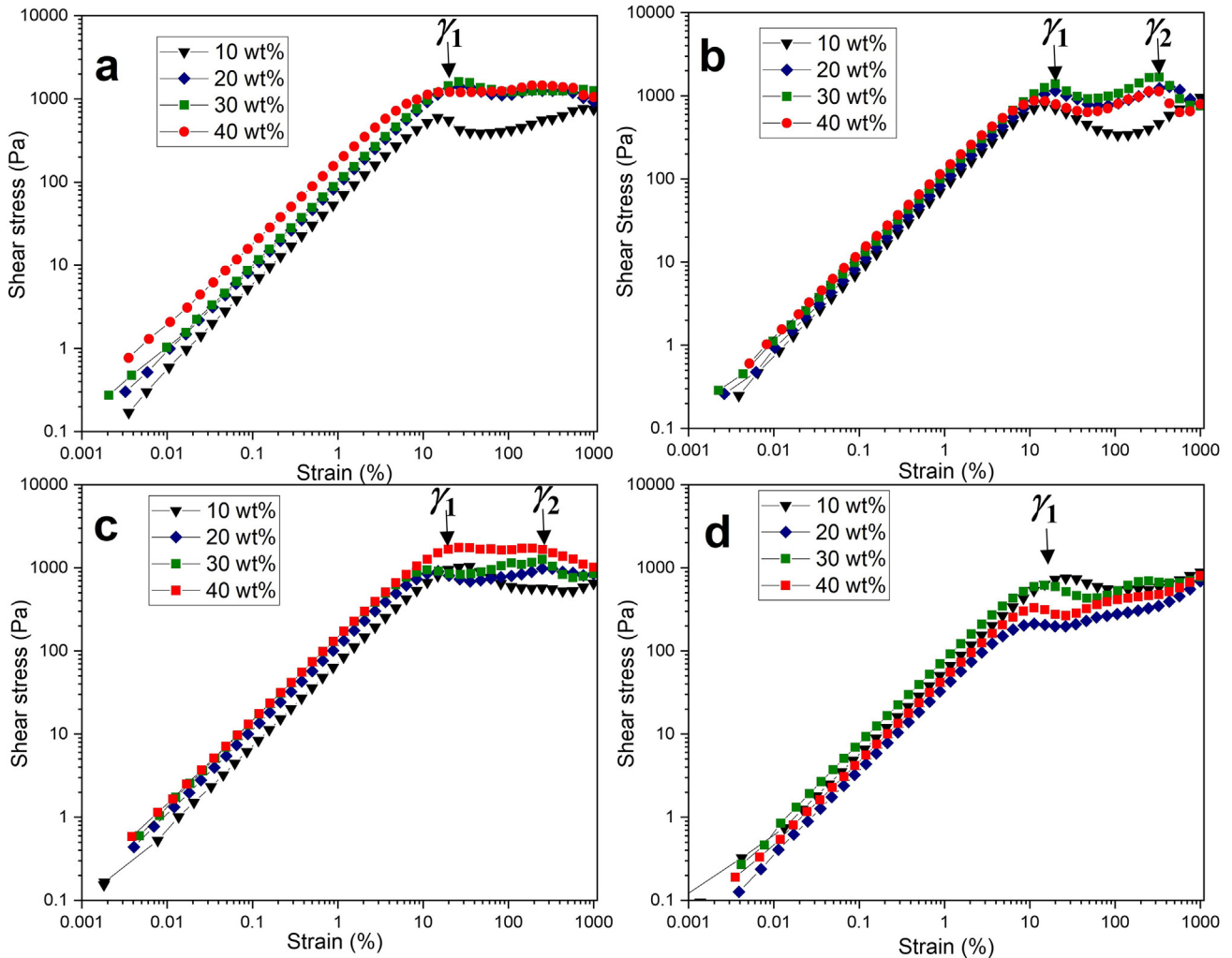
Fig. 4. Oscillatory strain sweeps of same samples as Figure 3 but expressed as shear stress, σ, as a function of strain (%). Black arrows highlight the first yielding γ_1 and the second yielding point γ_2, which represents the stress overshoot at large strains that is clearly prominent across the different oleosome contents. (a) 0.005 g carrageenan/g oleosome; (b) 0.005 g alginate/g oleosome; (c) 0.01 g carrageenan/g oleosome; (d) 0.01 g alginate/g oleosome.

yield point describes the rupture of weakest links within oleosome clusters, hold together by electrostatic bridging (Zambrano and Vilgis, 2022).

Beyond the first yielding point (γ_1), 0.005 g/g alginate (Fig. 4b) starts deforming plastically, first showing a slight decrease in stress followed before a second hump (γ_2) at higher strains appears. The position of γ_2 becomes more prominent as oleosome content increases (> 20 wt%), as its position shifts to smaller values γ_2 as observed in Figure 5. Second-step yielding is likely reminiscent of the so-called cage effect, which relates to the difficulty of a droplet to move due to the crowd of surrounding droplets (Sciortino and Tartaglia, 2005). Following deformation, droplets pack each other and restrict their movement resulting in an increase of stress, which upon deformation leads to a further release of each droplet. The cage effect may result from breaking bridging bonds and droplet rearrangements, which cause oleosomes to be trapped inside a cage while still interacting with the hydrocolloids, which partially coat the oleosomes. However, due to structural differences, the polysaccharides adsorb differently on the

oleosome surface, thus influencing the cage effect. This effect will be discussed in more detail in the following sections.

As the oleosome content decreases (< 20 wt%), droplet particles movement is less restricted thus they have a more accessible free volume to rearrange. This is reflected in the increase of the position of γ_2 as the oleosome content decreases (Fig. 5). Nevertheless, the different structural arrangement of alginate and carrageenan rules the concentration dependence of γ_2, which determines the final breaking of the confinement by the density-determined cage. Similar confining and caging effects were also observed by Zhao *et al.* (2014) in macrogels produced by bridging mechanism. Although the study above corresponds to a different silica particle system and microgels, it still shows similar (universal) physics. The caging effect is most likely to occur the denser the system is, *i.e.*, higher volume fractions, where rearrangements are less likely to occur, leading to dynamic arresting of oleosome droplets.

The second-step yielding transition is also observed among the different oleosome contents at 0.01 g/g carrageenan (Fig. 4c), where carrageenan presents its optimum bridging
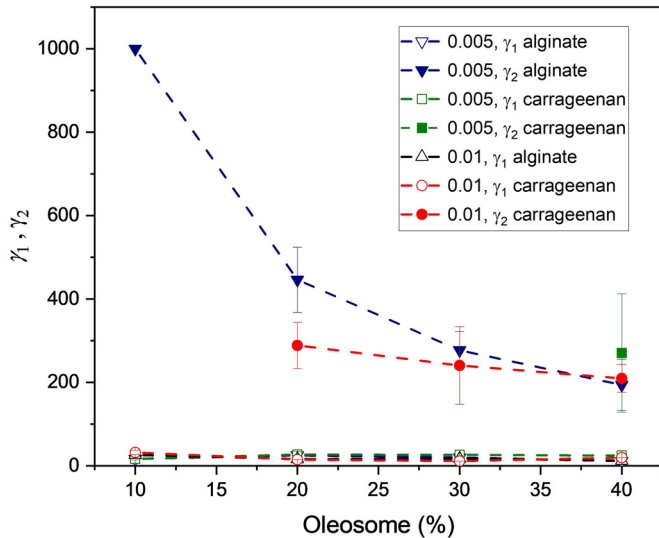
Fig. 5. Plots of γ_1 and γ_2 as a function of oleosome content (wt%) of alginate and carrageenan at two fixed polysaccharide/oleosome rations. γ_1 and γ_2 were extracted from Figure 4 as indicated by black arrows. Lines are guide for the eye.

ratio (Zambrano and Vilgis, 2022). However, the bumps corresponding to γ_2 do not appear as pronounced and sharp as 0.005 g/g alginate (Fig. 4b). This may be due to the weaker binding ability of carrageenan to oleosome surfaces compared to alginate, as suggested in a previous study (Zambrano and Vilgis, 2022). Similar observations were reported elsewhere where two-step yielding behavior was present in particle gel networks formed by high interparticle attraction (Pham *et al.*, 2008: Koumakis and Petekidis, 2011). In these systems, the overshoot on the yielding points becomes most pronounced in the samples with the strongest attraction.

On the contrary, 0.005 g/g carrageenan and 0.01 g/g alginate do not present a pronounced two-step yielding behavior. Instead, it is worth observing the deformation behavior upon γ_1. For 0.01 g/g alginate (Fig. 4d) at oleosome contents > 10 wt%, the stress response follows a slight shear thickening which may indicate single droplet and droplet clusters flowing around each other.

The previous study in bridging flocculated gels (Zambrano and Vilgis, 2022) indicated that at 0.01 g/g, the bridging ability of alginate is reduced, and instead, re-stabilization of oleosome droplets is taking place. However, due to the flexible confirmation of alginate polymer chains, alginate chains can still interconnect separate clusters. This might indicate that a transition between separate bridged clusters and stabilized single droplets is occurring.

On the contrary, the stress response in 0.005 g/g carrageenan upon γ_1 (Fig. 4a), at oleosome contents > 10 wt%, follows a plateau indicating the presence of single droplets which do not rearrange upon further deformation upon yielding of bridging bonds. It was previously demonstrated (Zambrano and Vilgis, 2022) that the chain conformation of ι-carrageenan is more effective for complete surface coating of oleosome droplets than for polymer bridging. As a result, it is more likely that upon deformation, γ_1, carrageenan chains will coat free single droplets than bridging other droplets. Due to

this characteristic, clusters in carrageenan–oleosome mixtures grow only until limited sizes. Similar transitions in bridging flocculated emulsions were found elsewhere (Dickinson and Pawlowsky, 1997). The flocculation transitions can range from a dispersion of separate clusters to a network of completely bridged droplets or stabilized-coated droplets plus separate clusters. As previously suggested, these transitions will depend on the type of polysaccharide and the ratio between polysaccharide chains and droplets.

Based on a previous study (Zambrano and Vilgis, 2022), the strong binding between alginate polymer chains and oleosome droplets at 0.005 g/g led to denser and more compacted clusters among the conditions evaluated. Polymer bridging will thus produce clusters, which depending on the strength of binding between polymer chains and the oleosome surface, will result in more compact or less compact structures. These will influence how it responds to deformation on a microstructural level. Deformation will yield either in one stage only: bridging bonds that are broken at a lower strain, or in two stages: bridging bonds that are broken at a lower strain; then, at a higher strain, cages are opened, and then the structure breaks up into before rearranged under given shear.

It is clear from comparing the structures of alginate and carrageenan that alginate can function as a bridging agent more effectively than the latter. Both polysaccharides consist of a structural backbone with flexible polymer chains. However, they differ in how their charges are distributed along the chain. Alginate backbone consists of mannuronic acid and guluronic acid groups arranged in blocks, whereas carrageenan presents a repeating galactose unit along its chain. Charge groups concentrated in blocks can promote stronger adsorption onto the surface of the oleosome droplets, as opposed to uniformly distributed charges along the carrageenan chain, which may lead to the less efficient use of some charges for interconnecting other droplets.

3.4 Final discussion

The experimental observations and interpretations propose a deeper understanding by using physical principles. Indeed, the behavior at larger strains is ruled by the different architecture of the two hydrocolloids (Zambrano and Vilgis, 2022). As mentioned, ι-carrageenan can be viewed as a weak but uniformly negatively charged polyelectrolyte, whereas alginate consists of (stiff) charged and uncharged flexible blocks. Therefore, alginate chains can form topological restrictions, *e.g.*, entanglements between the uncharged flexible blocks, as shown in Figure 6, which give rise to additional contributions to the viscoelastic behavior and the non-linear shear behavior. Uniformly charged polyelectrolytes such as carrageenans repel each other intensely and cannot form entanglements under these experimental conditions but are rather tightly adsorbed on the positively charged oleosome surfaces. The main differences are depicted in Figure 6.

From this model, it is reasonable to suggest that the chains can be easily exchanged between oleosomes under nonlinear shear at large deformations without changing much of the (free) energy of the system. On average, a similar number of monomers will be adsorbed on the oleosomes. For ι-carrageenan, it appears sufficient to describe the restriction of
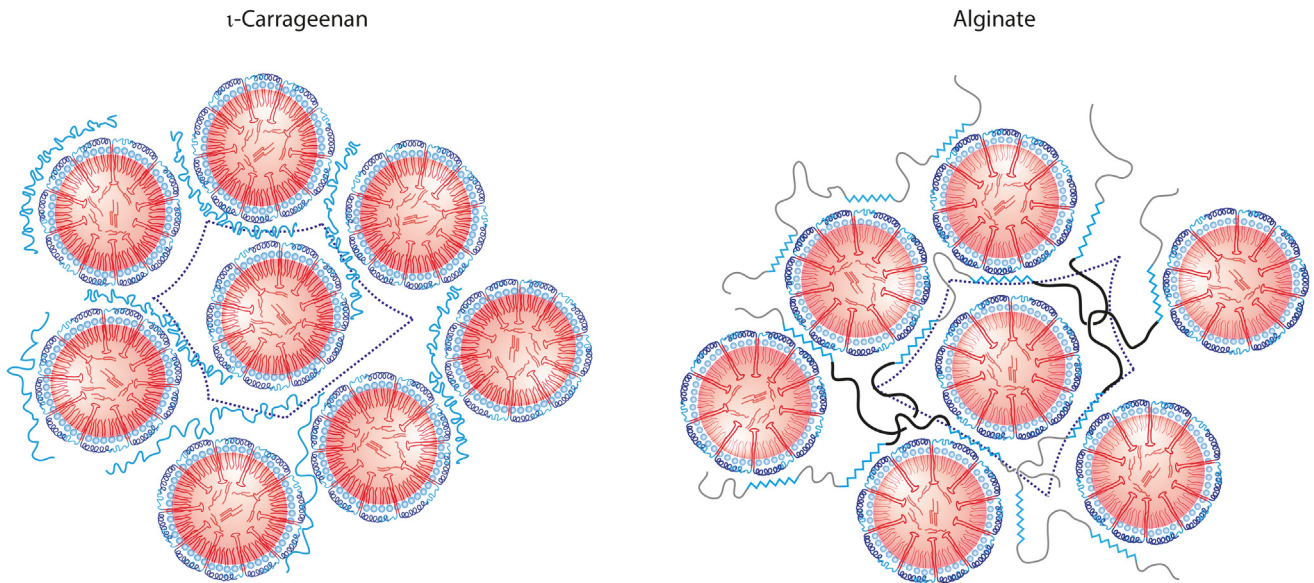
ι-Carrageenan   Alginate

Fig. 6. Schemes representing the different structures of oleosomes at high densities. The cages (polygons in dashed lines) limits motion, in addition alginate chains form entanglements (shown in black color).
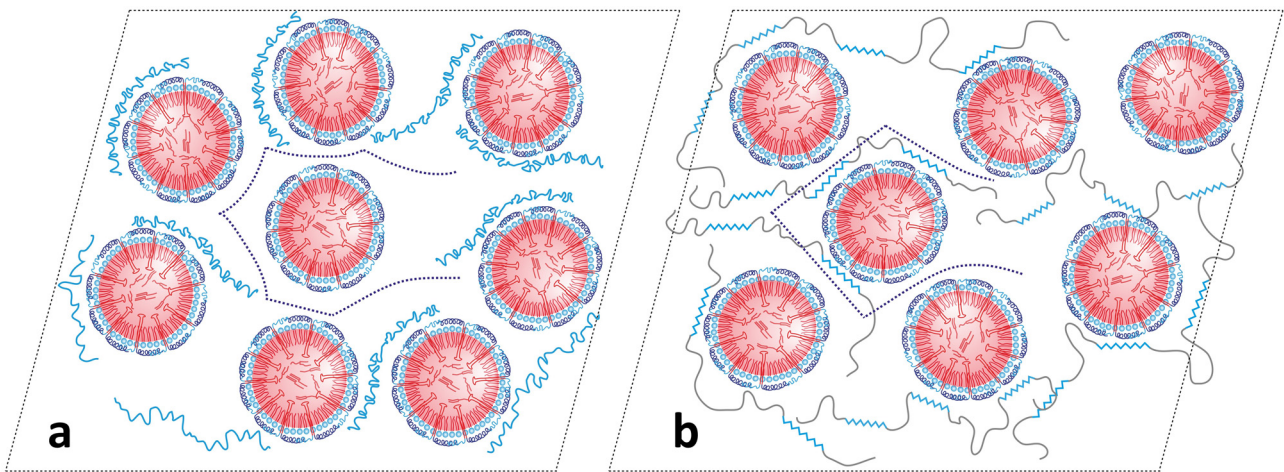

Fig. 7. Schematic representing oleogels cage under shear. (a) ι-carrageenan-oleosomes and (b) alginate-oleosomes.

motion of the oleosomes by a simple cage, which opens with hardly noticeable concentration dependency.

For higher carrageenan concentrations (0.01 g/g), a more pronounced second yield point becomes visible, which can be addressed to the higher repulsions of the chains which cannot adsorb at the time scales of the applied shear frequency (6.28 rad/s). When the cage opens under sufficiently high shear deformation, a plastic regime starts to show up, where the stress remains roughly constant by increasing deformation (see Fig. 7).

The pronounced second yield point in 0.005 g/g alginate can be assigned to the entanglement network of the alginate (see Fig. 6). The cage, defined by the mean distance of the oleosomes in the corresponding matrix opens, but the charged blocks of the alginates are still strongly adsorbed at the surfaces of the oleosomes. Larger deformations increase the shear energy, which needs to be taken by the electrostatic energy by adsorption and the deformation of the

entanglement network of the alginate. When the shear energy becomes larger than the energy gain by adsorption, chains become removed from the surface, the shear stress gets lower, but the entanglement network still resist stress. Only when the network disentangles, the flow of the oleosomes enters a (short) plastic regime. These processes are expressed by the clear appearance of two concentration dependence yield points, γ_1 and γ_2. Only after complete disentanglement of the alginate network, both systems flow similarly at these high shear deformations, as can for example seen in Figures 4b and 4c.

4 Conclusion

This study investigated the mechanical properties of oleogels created by a polymer bridging mechanism using soybean oleosomes as templates and polysaccharides as

structuring agents. Compact self-supporting gels can be prepared upon centrifuging bridging flocculated oleosome emulsions induced by sodium alginate and ι-carrageenan. Moduli in the LVR are determined by the bulk density of the bridged clusters and are not significantly different among the samples. However, the rheological behavior at larger deformations showed a more distinctive pattern among the different samples. Deformation will yield either in one single yielding point, γ_1 representing breakage of weak bridging bonds at lower strains, or in a two-step yielding point, γ_2, which indicates oleosome droplets are trapped into a cage that immobilizes the movement of oleosome droplets; thus, causes shear stress to increase. This second-yielding point becomes more prominent depending on whether the different sample conditions lead to compact and dense clusters and expresses the different molecular structure of the hydrocolloids. The compactness of the gel depends on the different molecular structure difference of alginate and ι-carrageenan and on the optimum bridging ratio determined by ratio of polysaccharide and olesome concentrations. In addition, to yield the most compact and dense microstructures at its optimum bridging ratio, 0.005 g/g, alginate yielded the most prominent γ_2 because the entanglement network given by its flexible chains resist deformation, causing the shear stress to increase significantly compared to the other samples. At its optimum bridging ratio, 0.01 g/g, ι-carrageenan showed a pronounced γ_2, albeit not as pronounced as alginate. This can be attributed to its decreased bridging efficiency compared to alginate, which results in less compacted microstructures. Clearly, these experiments provide different directions on how the molecular architecture of hydrocolloids (and proteins) provides ways to design macroscopic functional properties of oleogels according to their use. Polymer bridging offers the potential for efficient use of resources by using relatively low amounts of polysaccharides and oleosome emulsion while resulting in a high yield of gels using low centrifugal forces. Furthermore, polymer bridging as a gelation mechanism can be expanded to a wide range of polymers, including those with different structural conformations, sizes, and even emulsion droplets with a wide range of diameters and interfacial compositions. However, a more thorough fundamental understanding is needed to fully benefit from this method, for instance, the dynamics of bridging flocculation, such as the impact of polymer chain relaxation as a function of stirring speed or the mechanisms that underlie the optimum bridging ratio between polymer and droplets.

References

Ahn Y, Kim H, Kwak S-Y. 2019. Self-reinforcement of alginate hydrogel *via* conformational control. *Eur Polym J* 116: 480–487. https://doi.org/10.1016/j.eurpolymj.2019.03.017.

Dickinson E, Pawlowsky K. 1997. Effect of ι-carrageenan on flocculation, creaming, and rheology of a protein-stabilized emulsion. *J Agric Food Chem* 45(10): 3799–3806. https://doi.org/10.1021/jf970304d.

Huang AH. 1992. Oil bodies and oleosins in seeds. *Ann Rev Plant Physiol Plant Mol Biol* 43(1): 177–200. https://doi.org/10.1146/annurev.pp.43.060192.001141.

Koumakis N, Petekidis G. 2011. Two step yielding in attractive colloids: transition from gels to attractive glasses. *Soft Matter* 7 (6): 2456–2470.

Pham K, Petekidis G, Vlassopoulos D, Egelhaaf S, Poon W, Pusey P. 2008. Yielding behavior of repulsion-and attraction-dominated colloidal glasses. *J Rheology* 52(2): 649–676.

Piculell L. 2006. Gelling carrageenans. Food Polysaccharides and their applications (Vol. 239). 2nd ed. CRC Press. Boca Raton: Taylor & Francis Group.

Sciortino F, Tartaglia P. 2005. Glassy colloidal systems. *Adv Phys* 54 (6-7): 471–524.

Waschatko G, Junghans A, Vilgis TA. 2012a. Soy milk oleosome behaviour at the air–water interface. *Faraday Discuss* 158: 157–169.

Waschatko G, Schiedt B, Vilgis TA, Junghans A. 2012b. Soybean oleosomes behavior at the air–water interface. *J Phys Chem B* 116 (35): 10832–10841. https://doi.org/10.1021/jp211871v.

Yu X, Somasundaran P. 1996. Role of polymer conformation in interparticle-bridging dominated flocculation. *J Coll Interf Sci* 177(2): 283–287. https://doi.org/10.1006/jcis.1996.0033

Zambrano JC, Vilgis TA. 2022. Tunable oleosome-based oleogels: influence of polysaccharide type for polymer bridging-based structuring. *Food Hydrocoll* 137: 108399. https://doi.org/10.1016/j.foodhyd.2022.108399.

Zambrano JC, Vilgis TA. 2023. Tunable oleosome-based oleogels: effect of polysaccharide architecture on polymer-bridging based structuring. *Physics of Fluids* 35(2): 027131. https://doi.org/10.1063/5.0138206.

Zhao C, Yuan G, Han CC. 2014. Bridging and caging in mixed suspensions of microsphere and adsorptive microgel. *Soft Matter* 10(44): 8905–8912. https://doi.org/10.1039/C4SM01798F.