A Study of Syneresis and Stiffness in Biopolymers-Stabilized Emulsions Systems

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ABSTRACT

Syneresis in biopolymer structures such as low-moisture amorphous food and pharmaceutical systems is essentially controlled by thermodynamic factors and is regarded as a phase separation. The nature and structure of the components define the physical state and stability of the emulsions. In this study the ability of guar gum, sodium alginate, cyclodextrin, kappa-*, iota-,* and *lambda*-carrageenan to stabilize oil-in-water emulsions against syneresis was investigated. Triplicates emulsions containing one of these biopolymers were prepared using power ultrasound. The emulsions were stored at 4ºC and the extent of syneresis was assessed gravimetrically by measuring the amounts of exuded water from the emulsions. The biopolymers were compared on their ability to retarder syneresis. A possible relationship between time dependent-stiffness and the extent of syneresis was explored. The biopolymers were ranked on their ability to retard syneresis in the following order: guar gum, β-cyclodextrin, kappa*, iota, lambda* carrageenan and sodium alginate. Syneresis affects the structure of the emulsion by increasing the modulus of the emulsions. These results indicated that increase in stiffness is compatible with syneresis in emulsions or hydrogels. This information is critical for formulation, processing, and storage of food and drug systems susceptible to phase separation and syneresis.

INTRODUCTION

Many foods contain two immiscible phases, typically oil and water, as part of the components and they are mixed and stabilize to produce high quality, stable, and sensory appealing products that are commonly referred to as emulsions[1].

Conventionally, emulsions are classified as either oil-in water (O/W) emulsions in which oil forms the dispersed phase (oil droplets), and water forms the continuous phase (e.g., milk and sauces), or water-in-oil (W/O) emulsions in which water forms the dispersed phase and oil forms the continuous phase.

Syneresis is the term that describes liquid oozing out of food emulsions[2].

The mechanisms of syneresis are described using two common approaches, namely polymer and colloidal sciences. The first one uses theories and experimental work in polymeric solutions and gels. The second approach that applies to porous systems of particulate gels is based on the driving

force for particles rearrangement inside their gel structure[1]. Hence, syneresis is a phase separation that is formed due to the sudden removal of the aqueous phase correlated to the mobility of the polymer chains when the mobile fraction of the gel transforms into a frozen "glassy state" as a function of temperature, water content, and ion concentration[2]. This demarcation between gel and glass distinguishes between systems that synereses over long times and that do not[3]. Syneresis in emulsions or low-moisture amorphous food and pharmaceutical systems is mainly driven by thermodynamic instability between its components [4]

Thermodynamic instability causes formation of bonds between hydrophobic sites because water molecules in the micelles of the systems leave the structure of the dispersed phase. The nature and structure of the components, conditions such as temperatures, pressure, acidic or alkaline environments, and high ionic strength among other affect emulsion stability [4]. Therefore, choosing the appropriate emulsifier is very important for the stability of the emulsion.

Many emulsifiers commonly used in food applications include proteins, biopolymers such as hydrocolloids and their derivatives, and low-molecular weight surfactants. Emulsifier characteristics (type and concentration), bulk phase properties (interfacial tension and viscosity) and shearing conditions (pressure, number of passes, and instrument type) affect the characteristics of the oil droplets produced, as well as the bulk physicochemical properties of the emulsions[5].

Kinetically stable emulsions are formed using amphiphilic compounds that facilitate emulsion formation and improve emulsion stability[4, 5]. Amphiphilic compounds can form a thin coating around the oil droplets that inhibits their aggregation by generating repulsive forces between them, after the formation of droplets[6].

Food proteins have been used as emulsifiers in a variety of product formulations owing to their ability to facilitate emulsion formation. For example, proteins separated from bovine milk including whey protein isolate[7] ß-lactoglobulin, sodium caseinate, and bovine sodium albumin (BSA) are major proteins that have been used to improve emulsion stability[6].

Hydrocolloids are non-digestible hydrophilic biopolymers that usually have many hydroxyl groups and sometimes possess polyelectrolyte entities. They are derived from vegetables, animals, microbial, or synthetic origins and are naturally present in foodstuffs or added to regulate the functional properties of such materials They are also capable of forming gels or viscous solution[3]. Hydrocolloids are sometimes utilized in the manufacturing of milk products in food structuring, and in product stability improvement[6] as well as in cosmetics to overcome the unfavorable syneresis. In these roles, they function well only for structuring aqueous solvents but do not have sufficient molecular structure attributes to form strong bonds with hydrophobic oil. In a previous study[7] we investigated the stabilizing effect of κ-carrageenan and chitosan in nano encapsulates of vitamin K and Piroxicam. It was found that the columbic nature of carrageenan coupled with the amphiphilic character of whey protein permit a effective tailoring of the release of encapsulated substances.

It is well understood that the oozing out of water from emulsions through syneresis may affect the water content, and subsequently the water activity of the bulky fraction of the emulsions. Furthermore, the frozen "glassy state" resulting from syneresis may affect the physical state and rheological behavior of the bulky phase. This is mainly because foods with a glassy structure have a high modulus and viscosity and obviously their stiffness is high[9].

Peleg (1993, 1994) emphasized that any mechanical properties of foods and polymer systems related to stiffness can be characterized by a modulus curve showing downward concavity, and propose the following equation to model stiffness data:

$$\frac{Y}{Ys} = 1/\left\{1 + exp\left[\frac{T - Tc(W)}{a'(W)}\right]\right\} \qquad (1)$$

where Y is the stiffness parameter (e.g,, modulus), Y_s is the value of the stiffness parameter in a reference state (e.g., glassy state), $T_c(W)$ is temperature that characterizes the transition region, and a'(W) is a constant that indicates the steepness of the stiffness curve [(W) refers to the constant water content]

Equation (1) could be expressed also as (2)

$$\frac{Y}{Ys} = 1/\left\{1 + exp\left[\frac{W - Wc(T)}{a'(T)}\right]\right\} \qquad (2)$$

with value for water content, W, water content that characterized the transition region, $W_c(T)$, and constant a"(T) [(T) refers to the constant temperature] which is applicable in modeling stiffness as a function of water content.

The aim of this study is to investigate the effects of various biopolymers including guar gum, sodium alginate, cyclodextrin κ-carrageenan, ί-carrageenan, λ-carrageenan, and sodium alginate on their ability to stabilize oil-in-water emulsions against time-dependent syneresis. The main objective is to explore a possible relationship between syneresis and stiffness of the bulk of the emulsion brought about by changes in water content. The specific structure-properties relationship of the biopolymers and whey protein on the state and stability of the emulsion was analyzed to serve as resource for developing novel formulations in processing, storage or optimization of foods, nutraceuticals, and pharmaceuticals.

MATERIALS AND METHODS

Guar gum, *kappa*-carrageenan, gamma carrageenan, iota carrageenan, Na-alginate and whey protein are obtained from Ingredient Solutions, Inc, Waldo, Maine, USA. β-cyclodextrin, and tween 20 (surfactant) were from Sigma-Aldrich, MO, USA. Canola oil was purchased from a local grocery store. All these ingredients and the quantities used are given below in Table 3.

TABLE 1. List of ingredients used in the formulation of the emulsions given with specific quantities.

Ingredients	Quantity
Biopolymer	0.08g
Whey protein	3g
Canola Oil	10g
Tween 20	3 drops
Distilled water	Filled to 100mL

Triplicate emulsions were prepared as follows. About 0.08 g of the biopolymer was added to 10 mL of distilled water and softly heated while mixing until a clear solution was obtained. In a separate beaker 3 g of whey protein was dissolved into 40 mL of distilled water to obtain a solution. Then 10 mL of canola oil was added to 30 mL of distilled water and mixed using power ultrasound model UP 200S from Heilscher Ultrasound Technology, Ringwood NJ. The mixing was operated at 10 MHz for 10 minutes in an ice bath. During this preliminary mixing, 3 drops of tween-20 was added. The mixing was continued following a stepwise addition of the whey protein solution at 10 MHz for 10. Finally, the biopolymer solution was added to the mixture and subjected to a similar power ultrasound mixing to 5 min at a frequency of 5 MHz for 10 min. The total volume of the emulsion was adjusted to 100 mL using 0.02% w/v NaN_3 in distilled water and stored at 4ºC for 2h.

Study of time dependent syneresis

About 50 mL of triplicate emulsions were stored at room temperature in graduated cylinders and at time intervals of 48h, the volume of liquid separated out of the bulk of the emulsion was measured. Following the measurement conducted after 296h, the volume of exuded liquid was weighed and evaluated against the original weight of the emulsion samples. Dynamic mechanical analyzer (DMA) was used to determine G' for the solid's portions of the emulsion at the heating rate 1 °C/min from.

Emulsions characterization using Flow Cam Imaging

About 1 mL of emulsion was diluted 50 times in distilled water and placed in a Flow Cam cell for analysis using flash duration of 40,000 microseconds. Microparticles in the emulsions were visualized to evaluate particles dispersity in the emulsions.

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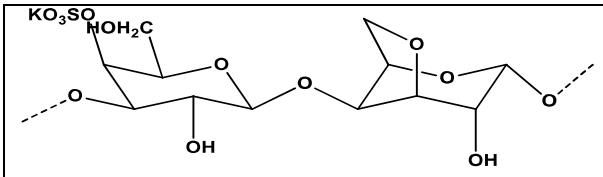
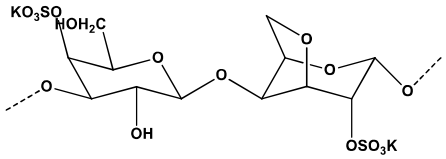
<td> 

a. Kappa-carrageenan</td>
<td> 

b. Iota-carrageenan</td>
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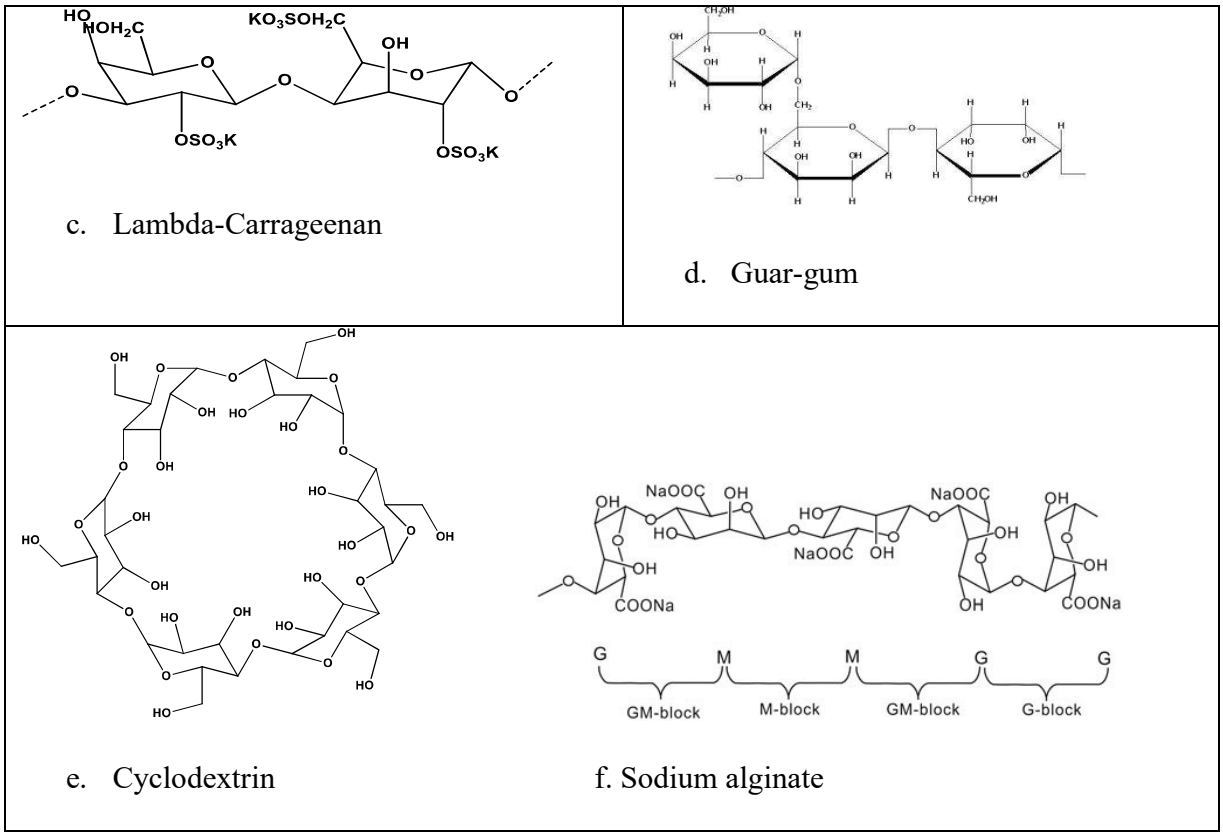

FIGURE 2: Structures of biopolymers ingredients studied.

RESULTS AND DISCUSIONS

All the emulsions prepared in this study were homogenous as shown in **Fig 3**. **Fig. 4** shows the time-dependent syneresis observed in the control emulsion (without biopolymer) as well as in sodium alginate and Lambda-carrageenan just 24h after the sample were placed for storage at room temperature. In opposite no loss of emulsion water was observed in the sample containing guar gum and cyclodextrin. Overall, in their ability to retard syneresis or phase separation, the biopolymers were ranked as follows: guar gum and, β-cyclodextrin *kappa, iota, lambda* carrageenan and Na-alginate. The interactions of components of the system and their physical and Guar gum chains possess galactomannans; mannose: galactose in the ratio of 2:1 that can be deprotonated if the pH increases. As we increase the pH of series of emulsions the [H^+] ion concentration keeps on increasing and as a result of which the neutral molecule becomes positively charged. Change in pH from acidic to basic causes changes in the interaction with the whey protein solution due to the reduction of repulsions with the particle. At the iso electric pH, guar gum-whey protein solution became highly unstable i.e., swelling might occur due to reduction of electric repulsions inside the protein-polymer matrix. Guar gum like β-cyclodextrin surged as very good inhibitors of syneresis. This could be explained by the fact that these biopolymers exhibit no electrostatic interactions with whey protein, the major central amphiphilic structurant. Hence, they

have no contribution to the packing of solid. They rather contribute to the dispersal of components and therefore, to increasing the entropy of the emulsion system.

FIGURE 3: Biopolymer-containing emulsion systems. All emulsions appeared homogenous just after they were prepared.

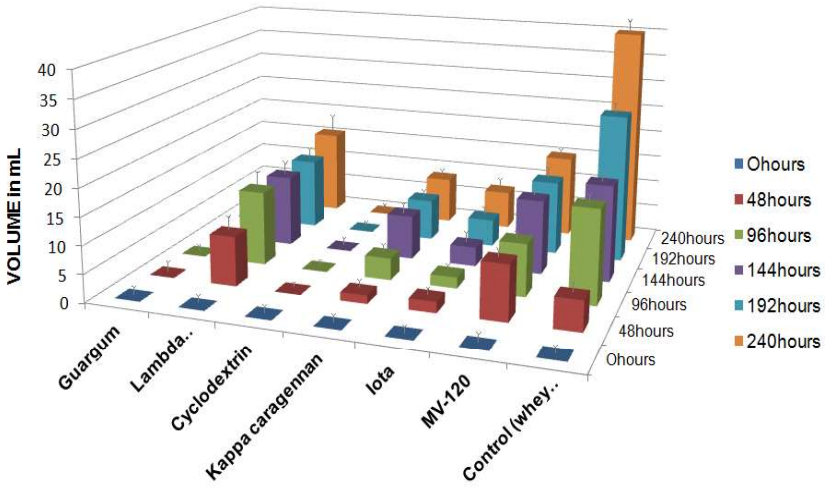

FIGURE 4: Time-dependent change on the volume of water lost through syneresis layer from biopolymer-containing emulsions.

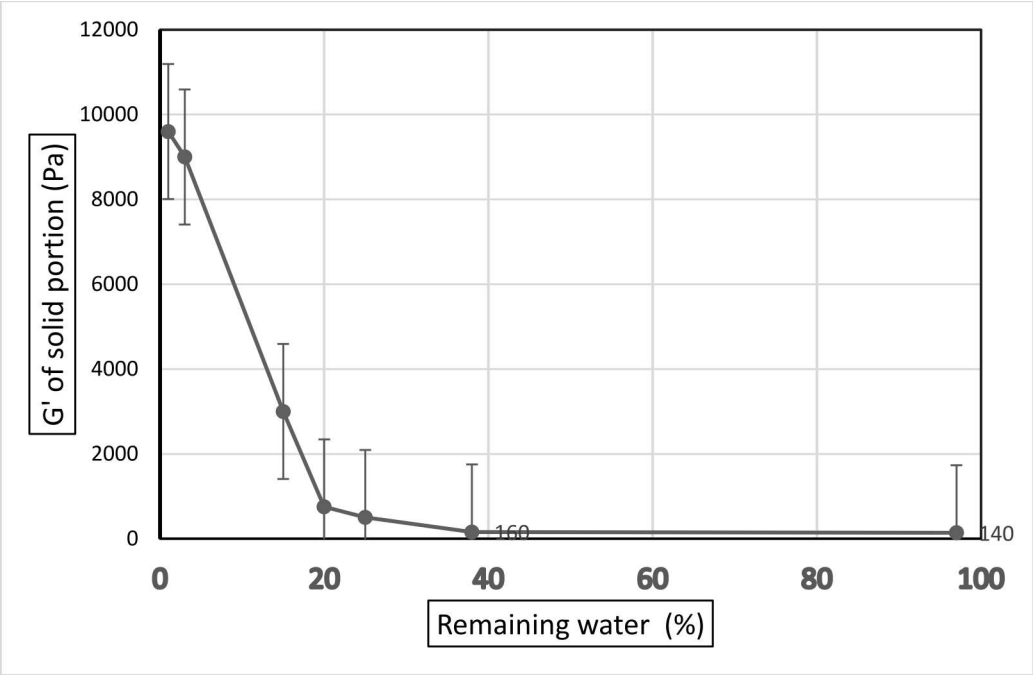

FIGURE 5: Master curve showing modulus of residual solids upon syneresis vs water content.

Fig. 5 depicts the effect of time-dependent syneresis on the storage modulus of the solid portion of the emulsion. The higher the droplet concentration, the slower the creaming rate. Increasing the droplet Concentratoon may not be a feasible solution to inhibiting gravitational separation in most food products, but it may be possible to introduce other non-fat particles to inhibit creaming by a similar mechanism[9]. Guar gum and cyclodextrin may exhibit essentially hydrogen binding with water molecules that may contribute to preventing them from being separated. In addition, they may inhibit creaming and excessive packing that generally forces water molecules out of the emulsion micelles.

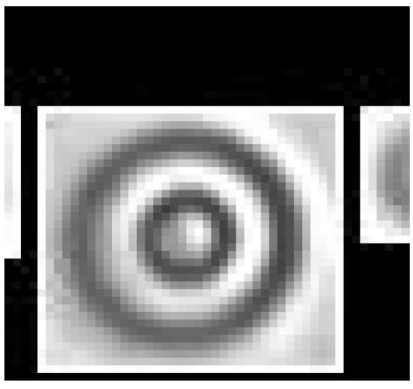

FIGURE 6. Flow Cam image of a microcapsule isolated.

Fig. 5 depicts a master curve of the storage modulus against water contents. This curse is in accordance with the trend predicted by Pelleg [11,12]. Syneresis occurring in the emulsions drains water out of the matrices making the emulsion to transition to a glassy like material characterized by a high modulus and high viscosity.

CONCLUSION

Control of syneresis or emulsion stability is important in the food industry in the creation of commercial products that maintain desired sensory attribute of foods and physicochemical properties throughout the products shelf life. There are various possible strategies available to retard emulsion instability by modulating the properties of the dispersed, continuous, and interfacial phases. The choice of the components is crucial to achieve this goal.

REFERENCES

1. Mizrahi, S. 2023. Chemical deterioration and physical instability in food and beverages. In Syneresis in food gels and its implications for food quality. Edited Skibsted, L. H. Cambridge, UK.

2. Hoefler A. 2004. Hydrocolloid sources, processing, and characterization. *Hydrocolloids* Eagan Press St Paul, Minnesota. 2004: 7-26. 13.

3. Mathias T, Carvalho JR, Carvalho C, Sérvulo EFC. Rheological characterization of coffee-flavored yoghurt with different types of thickener Caracterizaço reológica de iogurte sabor café com diferentes tipos de espessante. Alimentos e Nutriço Araraquara. 2012; 22: 521-529.

4. McClements, D. J. 2005. Food emulsions: Principles, practices, and techniques. Boca Raton, FL, USA: CRC Press.

5. Santana, R. C., Perrechil, F. A., & Cunha, R. L. 2013. High- and low-energy emulsifications for food applications: A focus on process parameters. *Food Eng. Reviews*, 5, 107–122.

6. Zinoviadou, K. G., Moschakis, T., Kiosseoglou, V., & Biliaderis, C. G. 2011. Impact of emulsifier-polysaccharide interactions on the stability and rheology of stabilised oil-in-water emulsions. Procedia Food Science, 1, 57–61.

7. Kouassi.G. K. Gogineni V, T. Gowda, N.M., Boley, M.S. and Koissi, N. 2013. Nano/microencapsulation of functional ingredients and drugs into biopolymer matrices: A study of stability and controlled release. In: Advances in Applied Nanotechnology for Agriculture, ACS Symposium Series 1143. *Ed.* Bosoon Park and Michale Appell. *Oxford University Press*.

8. Kouassi K. G. Teriveedhi, V., Milby, C., Tarab, A., Boley, S. M., et al. 2012. Nano/Micro-encapsulation of of linoleic acid in biopolymer matrices : effects of the physical state, water, and quercetin on oxidative and thermal stability. *J. Encapsul. Absorp. Sci.* 2. 1-10.

9. Chung, C., McClements D.J. 2014. Structure–function relationships in food emulsions: Improving food quality and sensory perception. *Food Structure*, 1. 106-126.

10. Roos, H. Y. 1995. Mechanical properties. In Phase transition in foods. Ed. Taylor, S. L. Academic Press, San Diego. 247-270.

11. Peleg, M. 1993. Mapping the stiffness -temperature-moisture relationship of solid biomaterials at and around their glass transition. *Rheol. Acta* 32: 575-1386.

12. Peleg M. 1994a. Mathematical characterization and graphical presentation of the stiffness-temperature relationship of gliadin. *Biotechol Prog*. 10. 652-654.