Comparison of Plant and Animal-Origin Proteins for the Impact of Maillard Conjugation on Nanoemulsion Formation and Stability

Sarah Caballero, Food Science and Technology

Mentor: Dr. Gabriel Davidov-Pardo

Kellogg Honors College Capstone Project

Background

- Nanoemulsions are colloidal systems of nanometric (d<500 nm) oil droplets dispersed in an aqueous phase.
- Valued in the food industry for their ability to create stable mixes of oil and water to incorporate lipophilic components.
- Biopolymers, especially proteins, can be used to stabilize emulsions due to their amphiphilic nature.
- Growing interest in legume-based proteins due to sustainability, low cost, allergen-free and vegan attributes.
- Protein-based nanoemulsions coagulate in pH ranges near the isoelectric point (pI) (pH=4.6), limiting food and beverage applications (Fig. 1).
- Maillard reaction covalently binds proteins and carbohydrates, which introduces steric hindrance between carbohydrate groups to reduce droplet coagulation (Fig. 2).

Objective

The goal of this research is to *assess the effectiveness of pea-protein or sodium-caseinate (control) Maillard conjugates* (P48h & C24h) as emulsifiers, as well as to compare the stability of PP-MC and C-MC emulsions at the *pI (pH=4.6)*, at various *temperatures*, and in exposure to different monovalent and divalent *salt concentrations*.

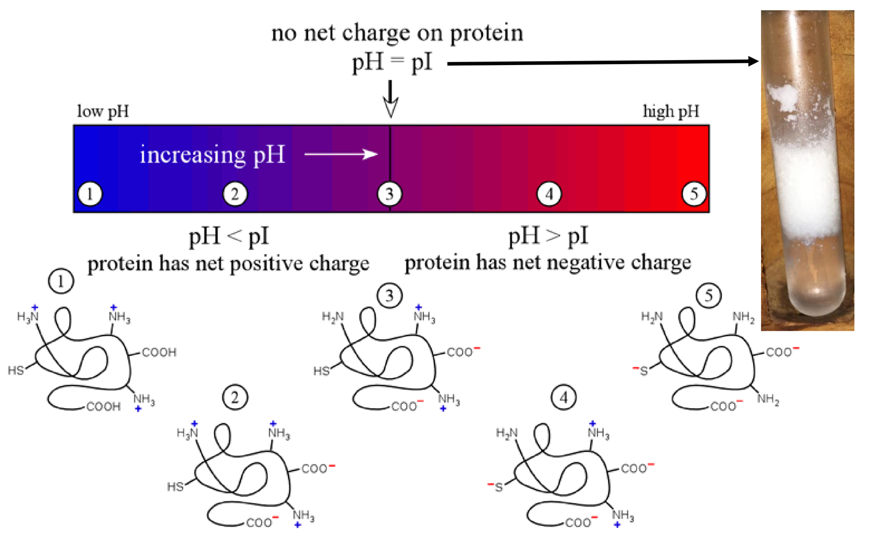


Figure 1: pH dependence of proteins; least solubility at pI (2).

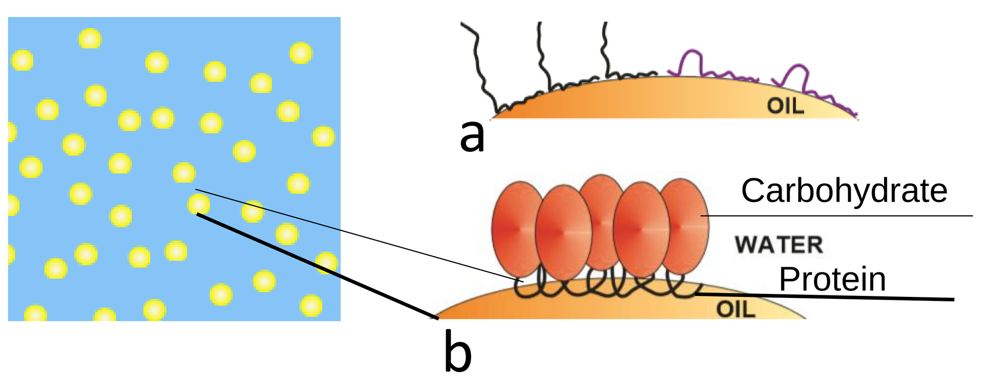
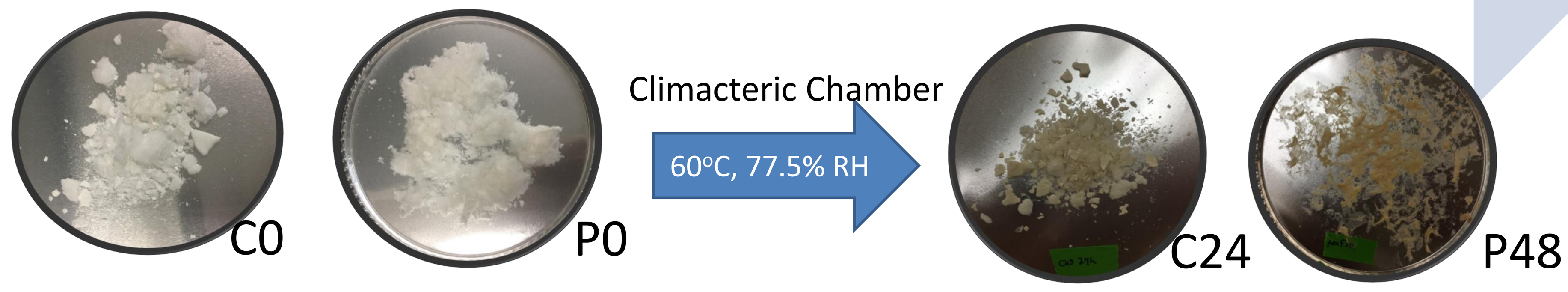


Figure 1: Schematic of oil-in-water emulsion a. Proteins adsorbing to interface and b. Maillard conjugates at interface (1).

Materials and Methods

Prepare Physical Mixture → Maillard Conjugation → Emulsion Formation → Particle Size Measurements → Stability Studies
- pH
- Temperature
- Ionic Strength



Prepare Physical Mixture (P0 & C0): Completely dissolved protein was mixed with equal-concentration 40kDa dextran in a 1:1 ratio. The mixture was then freeze dried (physical mixture).

Maillard conjugation: Freeze dried powder was put in a climacteric chamber at ***60°C, 77.5% RH*** for total time of 24 (caseinate) or 48h (pea protein). Protein conjugates were subsequently ground with a mortal and pestle and stored in a desiccator.

Emulsion formation: Selected emulsifier (either MC or physical mixture) was completely dissolved at 2% w/w in 5mM phosphate buffer (pH=7) by sonication treatment (intensity 4/10) for 10 minutes. The protein solution was mixed with medium-chain-triaclyglyceride (MCT) oil so that the ratio of surfactant to oil was 1:5. Coarse emulsions were subjected to high pressure homogenization at 30,000 psi for 5 passes.

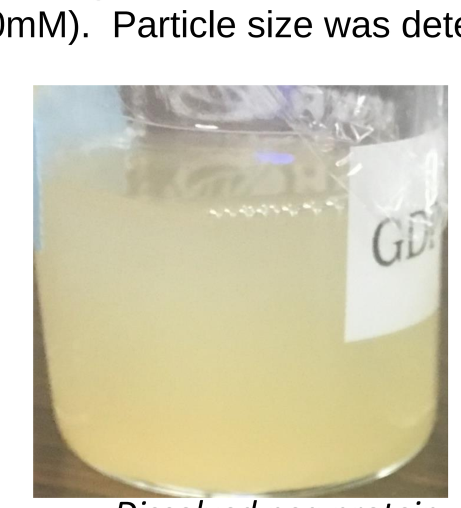
Particle size: Droplet diameter distributions of emulsions at pH=7 was determined by laser diffraction using a Beckman-Coulter LS 230.

Stability Studies

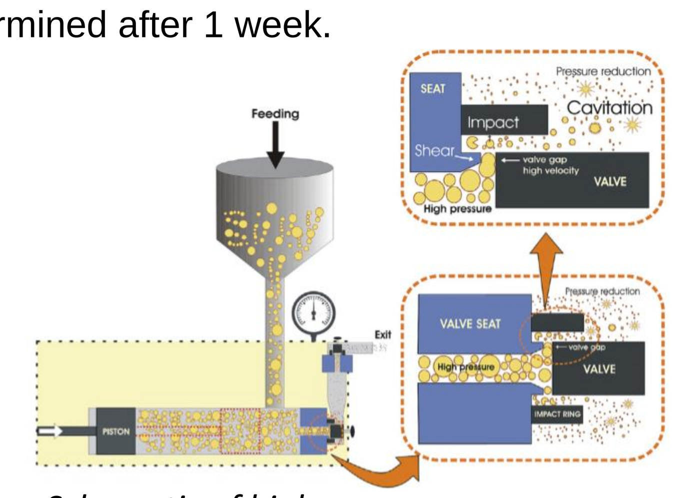
pH An aliquot of each emulsion was adjusted to the isoelectric point (pI) (pH=4.6) using HCl. The particle size distribution was again measured.

Temperature Nanoemulsions were incubated for 1 month at 4-55°C. Particle sizes were measured weeks 1,2,4.

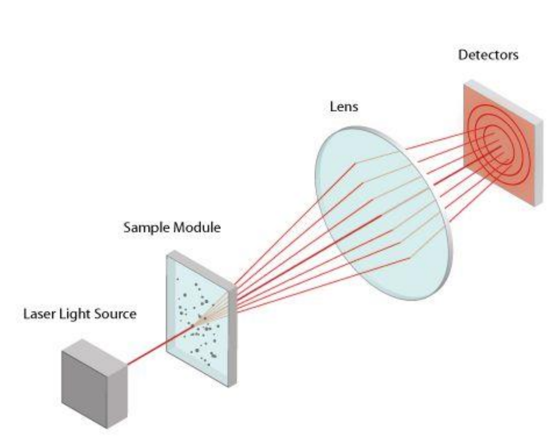
Ionic Strength Nanoemulsions were diluted with salt concentrations to final concentrations $CaCl_2$ (0-100mM) or NaCl (0-500mM). Particle size was determined after 1 week.



Dissolved pea protein Maillard conjugates



Schematic of high pressure homogenizer (1)



Schematic of laser diffraction (5)

Results and Discussion

	Pea 48h	Pea 0h	Cas 24h	Cas 0h
Nanoemulsion formation (Fig. 3)	More monomodal distribution	Bi- to tri- modal distribution	Monomodal, narrowest peak at small diameter	Monomodal
pH stability (Fig. 4-5)	Diameter increases, but stabilized (Fig. 5B)	Unstable	Highly stable (Fig. 5A)	Unstable
Ionic strength (Na^+) (Fig. 6)	Stable	Salting in/salting out (6), droplet growth	Stable	Stable
Ionic strength (Ca^{2+}) (Fig. 7)	Reversibly destabilizes	Unstable	Highly stable	Unstable
Temperature stability at pH=4.6 (Fig. 8-9)	Diameter increases for 4-25°C, destabilizes highly at 55°C (Fig. 9)	Unstable	Destabilizes at 55°C after 1 week and 37°C after 1 month (Fig. 8)	Unstable
Temperature stability at pH=7	Stable 4-37°C, diameter increase but stabilized at 55°C	Stable 4-37°C, progressive destabilization at 55°C	Stable	Slight droplet growth for 37-55°C

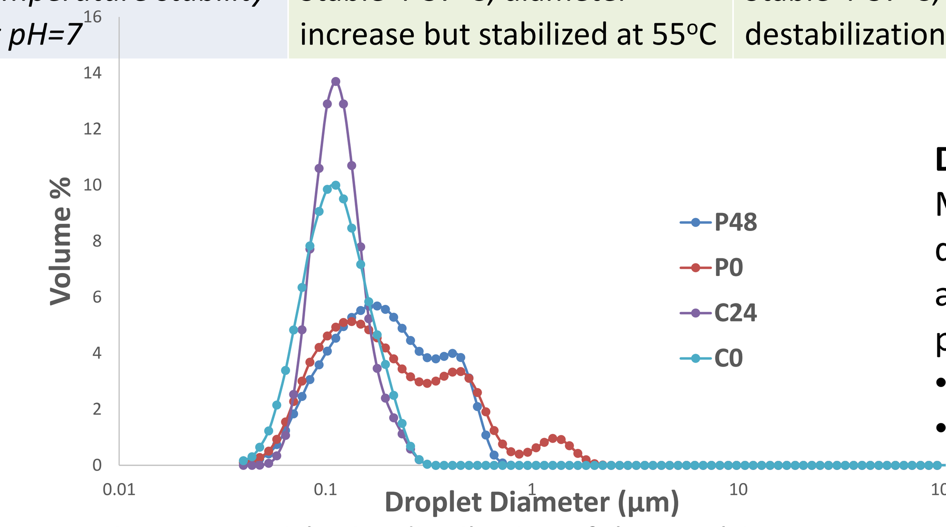


Figure 3: Particle size distribution of the emulsions.

Discussion:
Maillard conjugation increases monomodality and decreases size of nanoemulsions. Casein nanoemulsions are significantly more monomodal and narrow than pea protein nanoemulsions.
- Conjugated dextran increases emulsifier hydrophilicity
- More rapid absorption to droplet surface → smaller droplets (4)

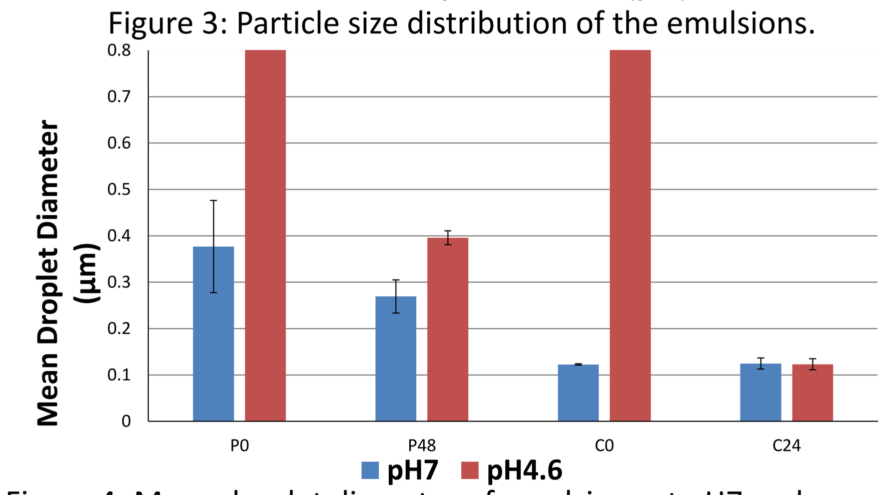


Figure 4: Mean droplet diameter of emulsions at pH7 and pH4.6.

Discussion: Steric hindrance from dextran groups (3)

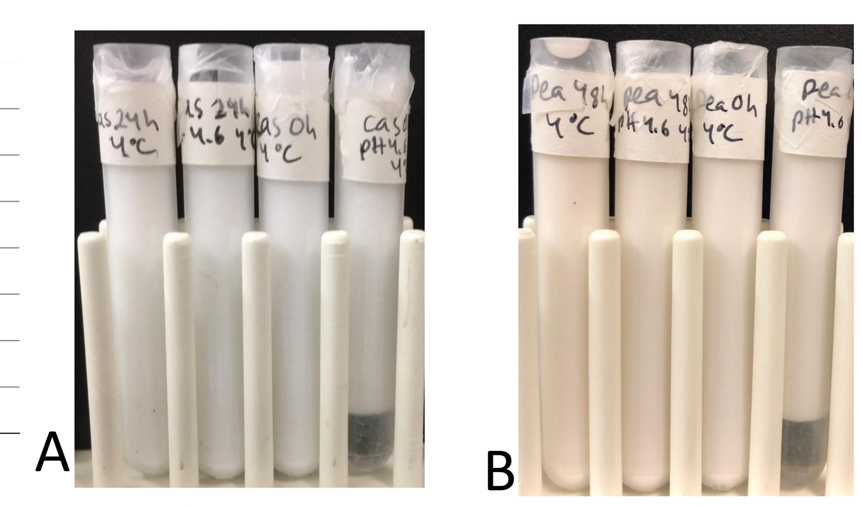


Figure 5: A) Casein nanoemulsions at 4°C and B) pea protein nanoemulsions at 4°C. L → R: MC, MC pH4.6, non-MC, non-MC pH4.6.

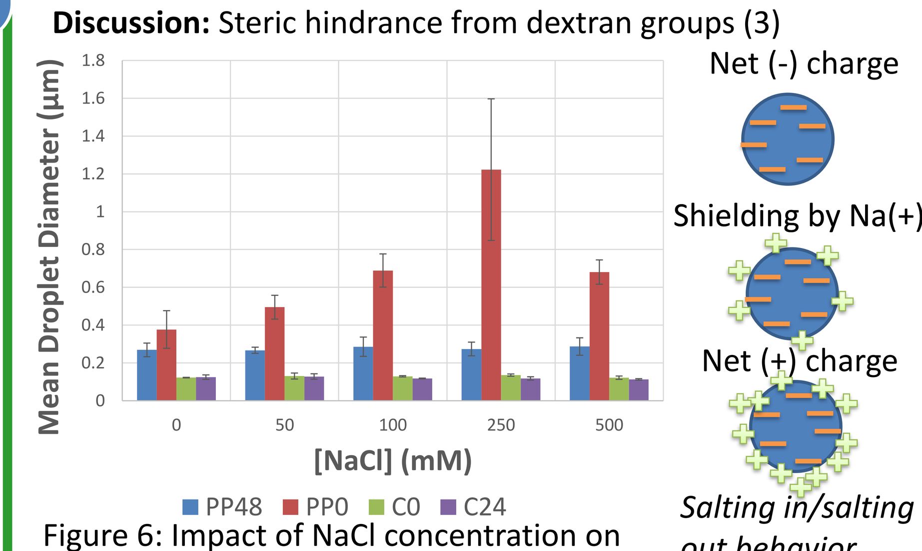


Figure 6: Impact of NaCl concentration on nanoemulsion stability.

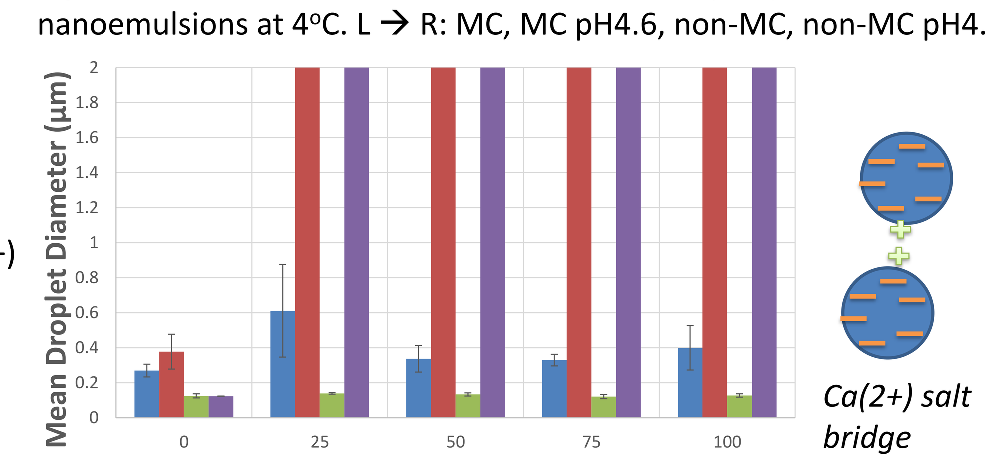


Figure 7: Impact of $CaCl_2$ concentration on nanoemulsion stability.

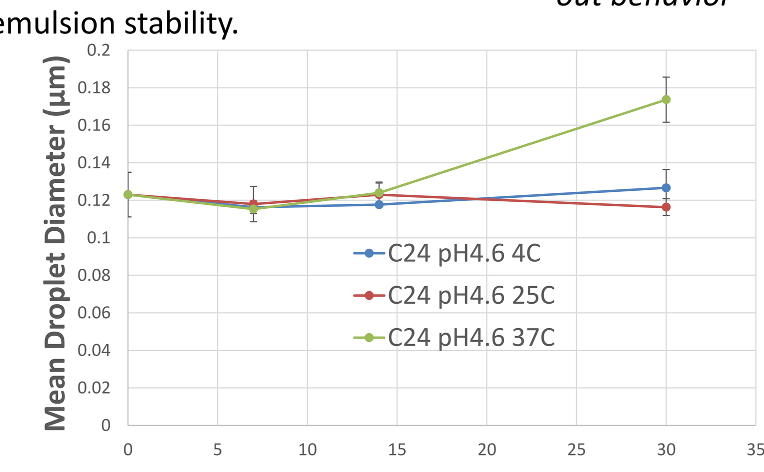


Figure 8: Impact of temperature on casein MC nanoemulsion stability at pH=4.6 during 1 month.

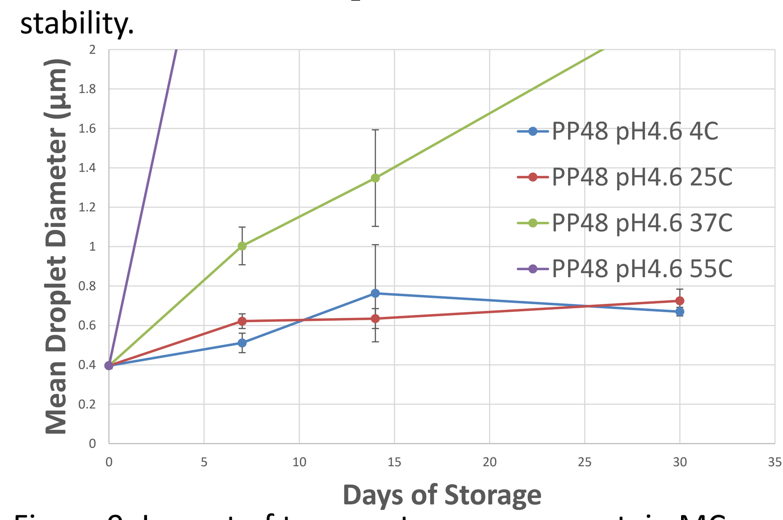


Figure 9: Impact of temperature on pea protein MC nanoemulsion stability at pH=4.6 during 1 month.

Conclusions

- Casein overall superior emulsifier than pea protein
- Maillard conjugation stabilizes pea protein-based nanoemulsions at isoelectric point
- MC of pea protein can withstand sodium and calcium concentrations
- Pea protein MC nanoemulsions stable at pH 4.6 at 4-25°C for up to 1 month
- MC improves nanoemulsion stability at high temperatures (>37°C)
- Pea protein MC can be used as emulsifiers in the food and beverage industry

References

1. Costa, Gomes, Andrade, & Cunha. (2017). Emulsifier functionality and process engineering: Progress and challenges. *Food Hydrocolloids, 68*, 69-80.
2. Cronk, J. D. (2016). Lecture 6 Primary Protein Structure. Retrieved from http://guweb2.gonzaga.edu/faculty/cronk/CHEM440pub/L06.html
3. Davidov-Pardo, G., Perez-Ciordia, S., Marin-Arroyo, M., & Mcclements, D. (2015). Improving Resveratrol Bioaccessibility Using Biopolymer Nanoparticles and Complexes: Impact of Protein-Carbohydrate Maillard Conjugation. Journal Of Agricultural And Food Chemistry, 63(15), 3915-3923.
4. McClements, D. J., & Gumus, C. E. (2016). Natural emulsifiers — Biosurfactants, phospholipids, biopolymers, and colloidal particles: Molecular and physicochemical basis of functional performance. *Advances in Colloid and Interface Science, 234*, 3-26. doi:10.1016/j.cis.2016.03.002
5. Particle Technology Labs. (n.d.). Laser Diffraction Testing. Retrieved from https://www.particletechlabs.com/index.php/analytical-testing/particle-size-distribution-analyses/laser-diffraction
6. Xu, H.-N., Liu, Y., & Zhang, L. (2015). Salting-out and salting-in: competitive effects of salt on the aggregation behavior of soy protein particles and their emulsifying properties. *Soft Matter*, 5926-5932.