Nanoemulsions are colloidal systems of nanometric (d<500 nm) oil droplets dispersed in an aqueous phase. 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 (pl) (pH4-6), limiting food and beverage applications. Maillard reaction covalently binds proteins and carbohydrates, which introduces steric hindrance between carbohydrate groups to reduce droplet coagulation.

**Objective**
The goal of this research is to assess the effectiveness of pea-protein or soy-protein-casein (control) Maillard conjugates (P48 & C24) as emulsifiers, as well as to compare the stability of P4C and C4C emulsions at the pl (pH4.6) at various temperatures and in exposure to different monovalent and divalent salt concentrations.

**Results and Discussion**

- **Nanoemulsion formation**: More monomodal distribution
- **pH stability**: Diameter increases, but stabilized at pH 5.8
- **Ionic strength (Na+)**: Stable
- **Temperature stability**: Diameter increases for 4°C (Fig. 8-9) unstable
- **Stability Studies**: More monomodal, narrowest peak
- **Maillard conjugation**: Conjugated dextran increases emulsifier hydrophobicity
- **More rapid absorption to droplet surface**: Smaller droplets

**Discussion**:
Maillard conjugation increases monomodality and decreases size of nanoemulsions. Casein nanoemulsions are significantly more monomodal and narrow than pea protein nanoemulsions.

**Materials and Methods**

- **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 climactic chamber at 60°C, 77.5% RH for total time of 24 (casein) or 48h (pea protein). Protein conjugates were subsequently ground with a mortar 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-triglyceride (MCT) oil so that the ratio of oil:water to oil was 1:3. Coarse emulsions were subjected to high pressure homogenization at 30,000 psi for 5 passes.

**Stability Studies**

- **pH**: An aliquot of each emulsion was adjusted to the isoelectric point (pl) (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 CaCl2 (0-100mM) or NaCl (0-500mM). Particle size was determined after 1 week.

**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**