**Nanoparticles from Resistant starch as a candidate for Pickering emulsion gels**

Apostolidis Eftychios1,2, Gerogianni Anastasia1, Paximada Paraskevi2, Anagnostaki Eysevia1, Mandala Ioanna1

*1Department of Food Science and Human Nutrition, Food Process Engineering Lab, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece*

*2School of Food Science and Nutrition, University of Leeds, Leeds, LS2 9JT, UK*

Emulsions are defined as liquid systems consisting of two immiscible liquids, mainly oil and water, where one is uniformly dispersed in the other as fine droplets1. These systems are thermodynamically unstable because the two phases eventually separate as a result of physical processes like gravity and aggregation. For this reason, the addition of compounds known as stabilizers are commonly used. Notably, there is a class of emulsions that can be stabilized by entire particles in the framework of replacing typical emulsifiers. These are Pickering emulsions, where solid particles are agglomerated and absorbed at the emulsion interface forming a strong mechanical layer3,4. Starch is the most common naturally occurring, bioavailable, and biodegradable polysaccharide-based emulsifier that is used for the creation of these type of emulsions5,6. Starch granules in their natural state cannot be used as stabilizers since they must be much smaller than the oil droplet in order to anchor it. For this reason, reducing the size of starch's particle enhances their stability over time7,8.

In the current study, nanoparticles from high amylose corn starch a special type of resistant starch (RS) were used as emulsion stabilizers. Specifically, the effect of starch size on o/w emulsion preparations of medium-chain triglycerides was further examined. Starch nanoparticles were created using a novel sequential three-step physical process that included hydrothermal gelatinization, nano-precipitation, and ultrasonic treatment. With the Dynamic Light Scattering (DLS) method, starch's size was determined through its hydroscopic diameter at 170 nm9. Subsequently, o/w emulsions with varying starch nanoparticle concentrations (1% w/v, 3% w/v, and 5% w/v) and oil content (1% v/v, 5% v/v, and 10% v/v) were prepared using ultrasonication. The 3-phase contact angle and the interfacial tension of the different combinations of nanoparticles and oil were studied. In parallel, the emulsion stability, droplet size, ζ-potential, and rheological properties were examined for 60 days of storage at 4 °C and 25 °C to elucidate the effects of time and temperature on the prepared samples. DLS and confocal microscopy were used to determine the droplet size of the emulsions, which was roughly ~220 nm, exhibited a spherical shape located around the droplet, and did not appreciably change over time. The emulsions that maintained their stability during storage, had a nanoparticle concentration of 3% and 5% w/v and oil 1% and 5% v/v, respectively. These findings suggest that starch nanoparticles might be a useful stabilizer that meets the demands of clean-label Pickering emulsion gels with a variety of food and other nutritional applications, including fat mimetics or egg replacers. Furthermore, they deepen our understanding on food hydrocolloids by clarifying the link between structure and function.

References

1. Tan, C. & McClements, D. J. Application of advanced emulsion technology in the food industry: A review and critical evaluation. *Foods* vol. 10 812 (2021).

2. McClements, D. J. Critical review of techniques and methodologies for characterization of emulsion stability. *Critical Reviews in Food Science and Nutrition* vol. 47 611–649 (2007).

3. Chevalier, Y. & Bolzinger, M. A. Emulsions stabilized with solid nanoparticles: Pickering emulsions. *Colloids Surfaces A Physicochem. Eng. Asp.* **439**, 23–34 (2013).

4. Sarkar, A., Zhang, S., Holmes, M. & Ettelaie, R. Colloidal aspects of digestion of Pickering emulsions: Experiments and theoretical models of lipid digestion kinetics. *Advances in Colloid and Interface Science* vol. 263 195–211 (2019).

5. Marefati, A. & Rayner, M. Starch granule stabilized Pickering emulsions: an 8-year stability study. *J. Sci. Food Agric.* **100**, 2807–2811 (2020).

6. Xu, T. *et al.* Characteristics of starch-based Pickering emulsions from the interface perspective. *Trends in Food Science and Technology* vol. 105 334–346 (2020).

7. Ge, S. *et al.* Characterizations of Pickering emulsions stabilized by starch nanoparticles: Influence of starch variety and particle size. *Food Chem.* **234**, 339–347 (2017).

8. Saari, H., Fuentes, C., Sjöö, M. & Rayner, M. Production of starch nanoparticles by dissolution and non-solvent precipitation for use in food-grade Pickering emulsions. *Carbohydr Polym* **157**, 558–566 (2017).

9. Apostolidis, E. *et al.* Production of nanoparticles from resistant starch via a simple three-step physical treatment. *Food Hydrocoll.* **137**, 108412 (2023).