**Formation of nanoparticles and gels from ethanol-pretreated whey proteins**

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Whey proteins can form aggregates, nanoparticles or even gels at high concentrations (>5.0% w/w). This study focuses on the effect of ethanol on the denaturation of whey proteins, while exploring their ability to form nanoparticles and gels after ethanol’s removal. The ability of the produced nanoparticles to stabilize oil-in-water (o/w) emulsions was also investigated.

Nanoparticles were produced at different ethanol concentrations (5–70% w/w) and low protein concentrations (1.0–2.0% w/w) by controlling pH and ionic strength. Gels were formed using whey protein solutions, previously treated with ethanol and heating (70 oC/60 min). Removal of ethanol was achieved by the means of freeze drying. Rheology, laser diffraction, and confocal laser scanning microscopy were used to characterize the created nanoparticles, gels, and emulsions.

At sufficient ethanol concentration (>20% w/w), environmental conditions (pH and ionic strength) played a crucial role in obtaining nanoparticles of different sizes and properties after the removal of ethanol. Laser diffraction analysis and confocal microscopy revealed that whey protein nanoparticles were effective in stabilizing o/w emulsions against coalescence for one month storage, which could be attributed to the particle adsorption at the oil-water interfaces. Dynamic rheological measurements showed that samples heated in the presence of ethanol formed thermo-reversible gels upon cooling to 5 oC and had a higher storage modulus (G'~ 300 Pa) than the corresponding samples without the pretreatment with ethanol (G'~ 0.0002 Pa). Covalent (disulfide bonds) and non-covalent bonds (primarily hydrogen bonds) were crucial for the particle size and gel formation. The results suggest that nanoparticles and gels produced with ethanol pretreatment may lead to the development of innovative food products with different physicochemical and rheological properties, due to the extensive denaturing effect caused by ethanol.