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Impact of alcohols on the formation and stability of protein-stabilized nanoemulsions

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ABSTRACT

Nanoemulsions are increasingly being used for encapsulation, protection, and delivery of bioactive lipids, however, their formation from natural emulsifiers is still challenging. We investigated the impact of alcohol on the formation and stability of protein-stabilized oil-in-water nanoemulsions prepared by high-pressure homogenization. The influence of different alcohols (ethanol, 1-propanol, and 1-butanol) at various concentrations (0–25% w/w) on the formation and stability of emulsions stabilized by sodium caseinate, whey protein isolate, and fish gelatin was investigated. The mean particle diameter decreased with increasing alcohol concentrations from 0 to 10% w/w, but extensive droplet aggregation occurred at higher levels. This phenomenon was attributed to enhanced protein–protein interactions between the adsorbed emulsifier molecules in the presence of alcohol leading to droplet flocculation. The smallest droplets ($d < 100$ nm) were obtained when 10% w/w 1-butanol was added to sodium caseinate-stabilized nanoemulsions, but relatively small droplets ($d < 150$ nm) could also be obtained in the presence of a food-grade alcohol (ethanol). This study demonstrated that alcohol addition might be a useful tool for producing protein-stabilized nanoemulsions suitable for use as delivery systems of lipophilic bioactive agents.

1. Introduction

There has been growing interest in the utilization of nanoemulsions as delivery systems to encapsulate, protect, and release lipophilic active agents due to their high encapsulation efficiency, optical clarity, good physical stability, and high bioavailability [1–4]. In general, oil-in-water emulsions are thermodynamically unstable systems that consist of spherical oil droplets dispersed within an aqueous continuous phase. By convention, emulsions with droplet diameters in the nanomeric scale (typically between 20 and 200 nm) are referred to as nanoemulsions [2,3,5]. In contrast, emulsions containing droplets with larger droplets are referred to as conventional emulsions or macroemulsions. Both nanoemulsions and macroemulsions are thermodynamically unstable systems because the free energy of the separated oil and water phases is lower than that of the emulsion itself [1,6]. As a consequence, these emulsions typically breakdown over time due to a variety of destabilization mechanisms, *e.g.,* creaming, flocculation, coalescence, and Ostwald ripening [1]. Nevertheless, they can be fabricated to remain metastable for a considerable period by adding appropriate stabilizers, such as emulsifiers, texture modifiers, ripening inhibitors, or weighting agents. Nanoemulsions should not be confused with microemulsions, which are another type of colloidal delivery system containing lipid nanoparticles [7]. Unlike nanoemulsions, microemulsions are thermodynamically stable systems, however, they typically require relatively large amounts of synthetic surfactants to fabricate them, which may limit their use for certain applications [8,9].

In general, two different approaches can be used to fabricate nanoemulsions: high-energy and low-energy approaches [1,10]. In high-energy approaches, droplet disruption is mainly achieved by generating large pressure differences within mechanical devices, such as high-shear stirrers, high-pressure homogenizers, or ultrasound generators [6,11,12]. In contrast, low-energy approaches rely on the spontaneous formation of small oil droplets at the boundary between the aqueous and organic phases under certain system conditions [2,13]. The main advantage of low-energy approaches is that they are simple and inexpensive to carry out and do not require the use of specialized homogenization equipment, however, the main disadvantage is that high levels of synthetic surfactant are often required. This limitation is similar to that associated with the formation of microemulsions, however, the total amount of surfactant required to form nanoemulsions by

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low-energy methods is still less than that required to form microemulsions.

The main objective of the current study was to establish the influence of small chain alcohols on the formation and stability of protein-stabilized nanoemulsions fabricated using a high-energy approach. Previous studies have examined a number of factors that influence the formation of nanoemulsions, such as homogenizer type, operating conditions, sample composition, and the physicochemical properties of the component phases [1,5,14]. Typically, the mean particle diameter decreases with increasing homogenization pressure and number of passes [15], and smaller droplets are produced using small-molecule surfactants than using polymeric surfactants [16,17]. Several studies have also focused on the role of oil and aqueous phase viscosities on droplet disruption within homogenizers [16,17].

Droplet breakup during homogenization can be described by the Taylor equation in systems with low droplet concentration and low continuous phase viscosity [16]:

$$r \sim \frac{\gamma}{\eta_c \dot{\gamma}} \quad (1)$$

where γ is the interfacial tension, η_c is the continuous phase viscosity and $\dot{\gamma}$ is the shear rate. This equation highlights the fact that a reduction in the interfacial tension plays a major role in the formation of small-sized droplets. The addition of alcohol to an aqueous phase is known to reduce the oil–water interfacial tension [18,19], and may therefore be a potential method of further reducing the size of the droplets in nanoemulsions produced by high pressure homogenization.

The aim of the present study was to examine the impact of various aliphatic alcohols (ethanol, 1-propanol, and 1-butanol) on the formation of oil-in-water nanoemulsions stabilized by food-grade protein emulsifiers, *i.e.* sodium caseinate, whey protein isolate, and fish gelatin. We hypothesized that smaller droplets would be produced during homogenization when alcohol was present in the aqueous phase due to the reduction in interfacial tension. Alcohols with different chain lengths were utilized to examine the influence of their molecular structure on nanoemulsion formation and stability, however it should be noted that only ethanol is suitable for utilization within the food industry.

2. Experimental

2.1. Materials

Cold water fish skin gelatin (#049K0050) was purchased from Sigma–Aldrich Co., (Steinheim, Germany). Its average molecular weight and p*I* value were reported to be approximately 60 kDa and pH 6, respectively. Whey protein isolate (#B180214) was donated by Arla Foods Ingredients (Viby, Denmark). The whey protein isolate contained ⩾92% protein, ⩽6% moisture, ⩽4.5% ash, ⩽0.2% fat, and ⩽0.2% lactose, according to the manufacturer's specification. Sodium caseinate (#L080512201) was purchased from Rovita GmbH (Engelsberg, Germany) and contained ⩾88% protein, ⩽6% moisture, ⩽4.5% ash, ⩽1.5% fat, and ⩽1% lactose, according to the manufacturer's specification. All proteinous emulsifiers were used without further purification. Miglyol 812 N, a medium chain triacylglyceride (MCT) mixture, was obtained from Sasol Germany GmbH (Brunsbüttel, Germany). It served as a model lipid in the oil-in-water emulsion. Absolute ethanol (purity = 100%, density ρ = 0.79 kg/m^3), 1-propanol (purity ⩾ 99.5%, density ρ = 0.80 kg/m^3) and 1-butanol (purity ⩾ 99.5%, density ρ = 0.81 kg/m^3) were obtained from Carl Roth GmbH & Co. KG (Karlsruhe, Germany) and VWR International GmbH (Darmstadt, Germany). Analytical grade hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Double-distilled water was used in the preparation of all samples.

2.2. Solution preparation

An aqueous emulsifier solution was prepared by dispersing 2% w/w sodium caseinate, whey protein isolate or fish gelatin powder in double-distilled water–alcohol mixtures (0–25% w/w ethanol, 1-propanol, 1-butanol) containing sodium azide (0.02% w/w) as an antimicrobial agent. All solutions were stirred at ambient temperature overnight to ensure complete hydration and then adjusted to a pH of 7.0 using 1 M HCl and/or 1 M NaOH.

2.3. Emulsion preparation

Oil-in-water emulsions were prepared by homogenizing 10% w/w lipid phase (MCT) with 90% w/w aqueous phase (2% w/w emulsifier, 0–25% w/w alcohol, pH 7.0). Protein–alcohol dispersions were mixed with MCT using a high-shear blender (Standard Unit, IKA Werk GmbH, Germany) for 2 min at 24,000 rpm. The coarse premixes were then passed through a high-pressure homogenizer (Avestin, Inc., Ottawa, Ontario, Canada) for different numbers of passes (1–10) at various homogenizer pressures (700–2000 bar).

2.4. Droplet size distribution

Particle size distributions and polydispersity indices were measured using a dynamic light-scattering instrument (Nano ZS, Malvern Instruments, Malvern, U.K.). Prior to analysis, the emulsions were diluted to a droplet concentration of approximately 0.005% v/v with an appropriate buffer to avoid multiple scattering effects. The instrument calculates the particle diameter by determining the time-dependence of the intensity of scattered light from oil droplets that move in the aqueous phase due to Brownian motion. In dilute systems, the size is then calculated from the diffusion constant using the Stokes–Einstein equation [20]. The instrument reports the mean particle diameter (*Z*-average) and the polydispersity index (PDI) ranging from 0 (monodisperse) to 0.50 (very broad distribution). Each measurement was made with three readings per sample.

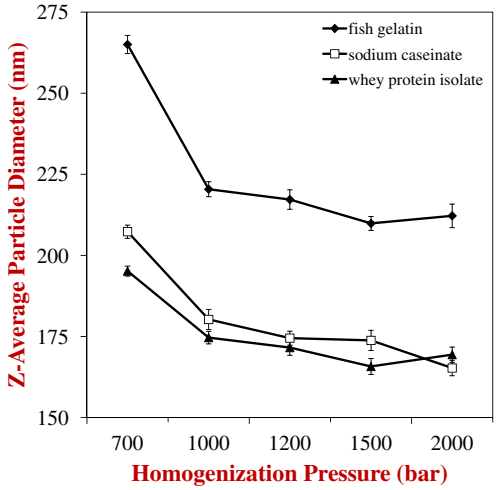

Fig. 1. Influence of homogenization pressure and protein type on **z**-average particle diameter of protein-stabilized oil-in-water emulsions after 5 passes through homogenizer: 10 wt% oil phase (MCT) and 90 wt% aqueous phase (2% w/w emulsifier, pH 7).