Nanoemulsion preparation by combining high pressure homogenization and high power ultrasound at low energy densities

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A B S T R A C T

Combinations of high pressure homogenization (HPH) and ultrasound (US) were studied as alternative processes to individual HPH and US to produce stable nanoemulsions, while reducing the energy requirement. A 15% (w/w) oil-in-water mixture containing 4.5% (w/w) of a blend of Tween 80 and Span 80 (1:1 w/w) was homogenized by means of combinations of HPH and US. In particular, 20 to 100 MPa HPH was applied before or after 20 or 60 s US, providing low and medium energy densities. Emulsions were analyzed for particle size distribution and mean diameter, viscosity and physical stability. Results were compared with those relevant to emulsions prepared by the application of individual HPH and US, providing comparable or higher energy densities. US and HPH applied in combination at low and medium energy density values allowed to obtain nanoemulsion having lower mean particle dimensions and, in most cases, higher stability than those prepared by using individual US or HPH at high energy densities. A greater efficiency was found for US preceding HPH.

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1. Introduction

High pressure homogenization (HPH) and high power ultrasound (US) induced changes of some physical and chemical properties of molecules are under study for exploitation at the industrial level for food processing and preservation purposes (Anese, Mirolo, Beraldo, & Lippe, 2013; Barba, Grimi, & Vorobiev, 2015; Donsì, Annunziata, & Ferrari, 2013; Fathi, Martin, & McClements, 2014; Floury, Desrumaux, & Legrand, 2002; Huang, Hsu, Yang, & Wang, 2013; Panozzo et al., 2013; Patrignani et al., 2013; Rastogi, 2011). Moreover, HPH and US have been proposed as alternative techniques to produce nanoemulsions (10–100 nm radius) since they can impart a sufficiently high energy input to reduce the droplet dimensions at nano-level of oil-in-water mixtures (Canselier, Delmas, Wilhelm, & Abismaïl, 2002; Abbas, Hayat, Karangwa, Bashari, & Zhang, 2013; Dumay, Chevalier-Lucia, Benzaria, Gracià-Julià, & Blayo, 2013; McClements, 2005; Silva, Cerqueira, & Vicente, 2012). The mean droplet diameter of an emulsion can be described as a function of energy density (E_v), that is the energy input per unit volume, by a power law (Stang, Schschmann, & Schubert, 2001; Schubert, Ax, & Behrend, 2003). Thus, the greater the emulsification efficiency the lower the droplet diameter. The efficiency of HPH to generate nanoemulsions mainly depends on the geometry of the homogenization valve, while in the US systems cavitation is the main effect. In HPH, the fluid is forced to pass in few seconds through a narrow gap in the homogenization valve, where it is

submitted to a rapid acceleration (Floury, Bellettre, Legrand, & Desrumaux, 2004; Floury, Legrand, & Desrumaux, 2004). The resulting pressure drop simultaneously generates intense mechanical forces, elongation stresses, cavitation and turbulence in the medium (Freudig, Tesch, & Schubert, 2003). Pressures between 50 and 150 MPa are generally applied to a coarse primary emulsion (Dumay et al., 2013; Solans, Izquierdo, Nolla, Azemar, & Garcia-Celma, 2005). During single pass process, a progressive decrease of the particle size can be obtained by increasing the homogenization pressure. However, up to a certain pressure level, which depends on the equipment design, particle size reduction is no longer expected (Floury, Desrumaux, & Lardiéres, 2000; Dumay et al., 2013; Jafari, He, & Bhandari, 2006; Lee & Norton, 2013; Quian & McClements, 2011). Multiple passes through the homogenizer are eventually applied to further reduce not only the mean particle diameter but also the width of the particle size distribution, and improve emulsion stability against coalescence (Cortès-Muñoz, Chevalier-Lucia, & Dumay, 2009; Floury et al., 2000; Quian & McClements, 2011). As a consequence an increase of energy requirement, that is proportional to the number of passes in the homogenizer, has to be expected.

In US, the energy is transferred to the fluid by the propagation of ultrasound waves in the frequency range of 20–100 kHz for a few seconds to several minutes (Abbas et al., 2013). These waves create alternate zones of compression and rarefaction, leading to development and subsequent collapse of microscopic cavitation bubbles. During collapse, intense shockwaves are created into the fluid, which are associated with high velocity gradients and shear stress. US emulsification is believed to occur mainly in the vicinity of the collapsing bubbles, where the

high fluid velocity causes the mixing of emulsion and droplet size reduction (Ashokkumar, 2011). The longer the treatment time the greater the droplet break-up (Abbas et al., 2013; Delmas et al., 2011), up to a threshold above which a further increase in residence time would not lead to a concomitant reduction of droplet diameter (Kentish et al., 2008; Leong, Wooster, Kentish, & Ashokkumar, 2009).

It is noteworthy that the application of HPH and US for nanoemulsion preparation at the industrial level is limited by several drawbacks. One major issue is relevant to high energy requirement to generate nanoemulsions. This implies the use of specially designed equipment, working at high pressures/number of passes or for long times during HPH and US, respectively, that indeed do not fit with industrial needs, such as continuous/uninterrupted flow, low energy consumption, low operating and maintenance costs, reduced replacement of wearing parts. Moreover, it cannot be underestimated that high intensity HPH and US processing may be responsible for undesired temperature increase (Abbas et al., 2013), that could negatively affect the product sensory and healthy quality. Thus, the possibility to decrease the energy requirement associated with HPH and US appears a hot topic in the attempt to reduce processing costs as well as increase the sustainability of food productions.

The aim of this work was to study technological solutions for nanoemulsion preparation to improve the homogenization process efficiency, while reducing the energy requirement and thus costs. To this purpose, a 15% (w/w) oil-in-water mixture containing 4.5% (w/w) of a blend of non-ionic surfactants (i.e., Tween 80 and Span 80 in 1:1 w/w ratio) was subjected to HPH and US that were provided in combination at low and medium energy density values. In particular, a single pass HPH, in the range of 20 to 100 MPa, was applied before or after 20 or 60 s US. Particle size distribution and mean diameter, viscosity and physical stability of the HPH-US and US-HPH treated samples were assessed and compared with those relevant to emulsions prepared by the application of individual HPH (up to 150 MPa; single or multiple passes) and US (up to 300 s), providing comparable or higher energy densities. This investigation is, to date, the first attempt to study the feasibility of HPH and US combined techniques in the light of reducing the energy requirement and costs associated with emulsification.

2. Materials and methods

2.1. Coarse emulsion preparation

The oil phase was prepared by dispersing 13.67% (w/w) sorbitan monooleate (Span 80, Tego SMO V, A.C.E.F. S.p.A., Florenzuola d'Arda — Piacenza, Italy) into commercial sunflower oil. The aqueous phase was prepared by mixing 3.04% (w/w) polyoxyethylene monooleate (Tween 80, Tween80®, Sigma Aldrich, Milano, Italy) with deionized water. The aqueous and oil phases were stirred separately at 20 °C for 30 min until the surfactants were completely dissolved. The coarse emulsion was prepared by mixing 17.05% (w/w) oil phase with 82.95% (w/w) aqueous phase using a high-speed blender (Polytron, PT 3000, Cinematica, Littau, Swiss) at 8000 rpm for 1 min. The oil and surfactants concentrations in the coarse emulsion were 15.00 (w/w) and 4.50% (w/w), respectively. The coarse emulsion was divided into two aliquots; the first one was taken as a control, while the other one was immediately subjected to the homogenization processes.

2.2. Homogenization processes

2.2.1. High pressure homogenization (HPH)

A continuous lab-scale high-pressure homogenizer (Panda Plus 2000, GEA Niro Soavi, Parma, Italy) supplied with two Re + type tungsten carbide homogenization valves, with a flow rate of 10 L/h, was used to treat 150 mL of coarse emulsion. The first valve was the actual homogenization stage and was set at increasing pressure up to 150 MPa. The second valve was set at the constant value of 5 MPa.

Additional samples were prepared by subjecting the coarse emulsion to HPH for up to 5 successive passes at 120 MPa. At the exit of the homogenizer, after the final pass, the nanoemulsions were forced into a heat exchanger (GEA Niro Soavi, Parma, Italy) and cooled to 20 ± 2 °C.

2.2.2. High power ultrasound (US)

An ultrasonic processor (Hieschler Ultrasonics GmbH, mod. UP400S, Teltow, Germany) with a titanium horn tip diameter of 22 mm was used. The instrument operated at constant ultrasound amplitude and frequency of 100 μm and 24 kHz, respectively. Aliquots of 150 mL of coarse emulsion were introduced into 250 mL capacity (110 mm height, 60 mm internal diameter) glass vessels. The tip of the sonicator horn was placed in the centre of the solution, with an immersion depth in the fluid of 50 mm. The ultrasound treatments were performed up to 300 s. At the end of each treatment, samples were cooled to 20 ± 2 °C in an ice bath.

2.2.3. Combined HPH and US

The coarse emulsion (150 mL) was subjected to HPH before or after US. The time between the two treatments did not exceed 30 s. Homogenization pressure was set at 20, 50, 80 and 100 MPa, while US treatments were applied for 20 and 60 s. Samples were cooled to 20 ± 2 °C at the end of the second treatment. In particular, the nanoemulsions were forced into a heat exchanger (GEA Niro Soavi, Parma, Italy) or cooled in an ice bath, depending on the final treatment, i.e. HPH or US.

2.3. Sample storage

After preparation, the nanoemulsions were collected and stored at 4 °C for up to 15 days.

2.4. Temperature measurement

The sample temperature was measured just before and immediately after (i.e. before the cooling step) each treatment by a copper-constantan thermocouple probe (Ellab, Hillerød, Denmark) immersed in the fluid, connected to a portable data logger (mod. 502A1, Tersid, Milan, Italy). In addition, during US, the temperature was recorded as a function of time, by immersing (50 mm) the thermocouple tip in the fluid, half way between the sonotrode and the inside wall of the vessel.

2.5. Energy density

The energy density (E_v, MJ/m^3) transferred from the homogenization valve to the sample was determined as described by Stang et al. (2001), according to Eq. (1):

$$E_v = \Delta P \qquad (1)$$

where ΔP is the pressure difference operating at the nozzles.

The energy density transferred from the ultrasound probe to the sample was determined calorimetrically by recording the temperature (T, K) increase during the homogenization process (Mason, Lorimer & Bates, 1999; Raso, Manas, Pagan, & Sala, 1999; Schubert et al., 2003). The following Eq. (2) was used:

$$E_v = \frac{mc_p(\partial T/\partial t)}{V} \times t \qquad (2)$$

where m is the sample mass (kg), c_p is the sample heat capacity (4.186 kJ/kg K), V is the sample volume (m^3), and t (s) is the duration of the emulsification procedure.

The energy density of multiple passes HPH and combined treatments was calculated as the sum of the energy density values of the corresponding single pass HPH or US plus HPH treatments.