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Ultrasonic preparation of stable flax seed oil emulsions in dairy systems – Physicochemical characterization

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ABSTRACT

This study reports the incorporation of 7–21% of flax seed oil in pasteurized homogenized skim milk (PHSM) using high intensity ultrasound (US) at 20 kHz between 1 and 8 min and at varying power levels. A minimum process time of 3 min at an applied acoustic power of 176 W was sufficient to produce emulsion droplets (7% oil) with an average mean volume diameter of 0.64 μm and they were stable at least 9 days at 4 ± 2 °C. The mechanical, cavitational and cavitation-after-effects of US are responsible for the production of smaller sized emulsion droplets and process-induced modifications of milk proteins. A very small proportion (less than 20%) of partially denatured whey proteins provided stability to the emulsion droplets. The emulsion droplets also showed a surface potential of about −30 mV due to the adsorbed proteins, which provided further stability to the emulsion droplets due to electrostatic repulsion. In order to see if other high shear techniques can generate stable emulsions, experiments were carried out using Ultraturrax (UT) at similar energy densities to that of US. UT did not produce stable emulsions until 20 min of processing suggesting the superiority of US emulsification process.

1. Introduction

Consuming healthy food has become a major trend in the last decade leading to the development of novel food processing techniques for the encapsulation and delivery of bioactives/nutraceuticals. Most bioactives and nutraceuticals are hydrophobic compounds. A poor water solubility of these compounds causes enormous difficulties in delivering them in food. The delivery of such oil-based bioactives/nutraceuticals as emulsions is well-known (Garti & Yuli-Amar, 2008; Couedelo et al., 2011). Conventionally, food emulsions (O/W) are obtained using high shear mixtures such as UT and piston homogenizers with the assistance of emulsifiers and stabilizers to achieve considerable emulsion stability upon storage (Dapcevic Hadnadev, Dokic, Krstonosic, & Hadnadev, 2013; Maali & Mosavian, 2013; Santana, Perrechil, & Cunha, 2013). The use of large quantities of emulsifiers and Non-GRAS (Generally Recognised As Safe) additives are not permitted in foods, this makes the food industry to rely only on a few range of emulsifiers and this poses a huge challenge in the area of new product development.

US has been used for creating emulsions in foods (Soria & Villamiel, 2010; Wulff-Perez, Torcello-Gomez, Galvez-Ruiz, & Martin-Rodriguez, 2009). Much of the existing research work in the area of ultrasonic emulsification has focussed mainly on simple matrix such as an emulsion of sunflower oil in water. The delivery of bioactives in a complex/real food matrix (health beverage) using US remains as a vast area to be explored. Unlike simple matrices, a complex food matrix is composed of proteins, carbohydrates, fat, water, vitamins and minerals. Few studies have identified the use of emulsifiers/surfactants in production of smaller oil droplet (Jafari, He, & Bhandari, 2006; Kentish et al., 2008; Leong, Wooster, Kentish, & Ashokkumar, 2009), however the stability during the storage of the product has not been studied in detail. In addition, formation of emulsions ultrasonically without the use of external stabilizers and emulsifiers (food additives) also remains unexplored. Hence, the purpose of this study is to deliver stable emulsions of a hydrophobic bioactive compound in a complex food matrix such as milk using ultrasound.

In the past, some studies have reported the preparation of soy oil emulsions (O/W) using milk fat globular membrane (MFGM) as an emulsifier and by employing high pressure homogenization (HPH) and microfluidizer (Corredig & Dalgleish, 1998; Roesch, Rincon, & Corredig, 2004). Biasutti, Venir, Marchesini, and Innocente (2010) have produced 15% O/W model dairy emulsions using milk cream and emulsifiers by HPH. However, the milk cream

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by itself is rich in MFGM (Harjinder, 2006) and the storage stability of these model emulsions was not discussed. In our study, we were interested in loading a significant amount of flax seed oil (7–21%) in pasteurized homogenized skim milk (PHSM) without using MFGM and other food additives. The incorporation of flax seed oil in PHSM by employing high intensity US has not been reported in the literature.

Flax seed oil is a widely popular non polar bioactive among the functional food category. It is obtained from flax seed (*Linum usitatissimum* L.) and is a rich source of omega-3-fatty acid, α-linolenic acid (ALA) (Carter, 1993; Mantzioris, James, Gibson, & Cleland, 1994). ALA is an essential fatty acid and is known to support cell, nerve & cognitive skills development in children and cardiovascular functions in humans (Joshi et al., 2006; Mazza, Pomponi, Janiri, Bria, & Mazza, 2007). In 2002, the Food and Nutrition Board of the US (Institute of Medicine) has recommended the adequate intake (AI) levels for ALA in adults (19 years and older): 1.6 g/day for men and 1.1 g/day for women to avoid any deficiency which will result in symptoms like scaly dermatitis. In addition, Mazza et al. (2007) have indicated a safe and effective dose of flax seed oil and are 3–6 g/day to prevent and treat neurodegenerative disorders.

Broadly, the aim of our study was to emulsify 7–21% of flax seed oil (59.9% ALA) in PHSM using US at 20 kHz under various experimental conditions. The stability of the emulsions was characterised by a number of techniques and compared with UT emulsification process.

2. Materials and methods

2.1. Materials

Fresh PHSM was purchased from a local supermarket and immediately stored at 4 °C until further use. The composition of the milk was 3.5% protein, 0.1% fat, and 4.9% lactose as labelled by the manufacturer. The manufacturer's specification was cross-checked in our lab for the proteins. The protein content was 3.46% by Bradford Assay. We did not observe any creaming-off in the PHSM sample for about 10 days of storage at 4 ± 2 °C indicating that fat content was very low.

Ultra pure (MilliQ) water was used in all experiments. Unrefined organically grown cold pressed flax seed oil with 59.9% of ALA was a gift sample from Stoney Creek Oil Products Pty Ltd, Australia.

2.2. Emulsification by US and UT

The emulsion composition was 7% flax seed oil (v/v) in 93% PHSM, unless mentioned otherwise. Both the water and oil phases were added sequentially to the water jacketed glass vessel and the sonicator horn was positioned at a depth of 0.3 ± 0.1 cm. Emulsions were obtained as 50 ml aliquots using a 20 kHz, 450 W ultrasonic horn (12 mm diameter, Branson Sonifier, Model 102 (CE)) at 88, 132 and 176 W of nominal applied powers (NAP) for different processing times from 1 to 8 min. During sonication, thermostated water was circulated continuously through a jacket surrounding the sonication cell and the water temperature was maintained at 22.5 ± 2 °C. The emulsified samples were stored in a refrigerator for about 9 days at 4 ± 2 °C. The analysis and storage studies were performed on both fresh and stored samples. In this paper, flax seed oil/milk, flax seed oil/water and oil/water emulsions are referred to as OM, OW and O/W, respectively. The term "unstable emulsion" refers to the system where the oil phase separated within 3 h of standing at room temperature whereas "good emulsion" refers to the system where the oil phase did not separate until 2 days and "stable emulsion" refers to the system where the oil phase did not separate until at least 9 days of storage at 4 ± 2 °C, respectively.

UT emulsions were prepared in the same jacketed vessel using Ultraturrax (IKA-Labortechnik) at speed dial value 4; 17,500 RPM (22.5 ± 0.5 °C) and at an energy density equivalent to that of US (discussed in Results section).

2.3. Particle size and zetapotential measurements

The particle size of the OM emulsion was measured on fresh and stored samples using a laser diffraction method by Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, U.K). A few droplets of the emulsion were suspended directly in recirculating water (1250 rpm, obscuration (14–16%) and refractive index of flax seed oil 1.475). The volume size distribution values viz., D(4,3), Dv90 and Dv50 were recorded. D(4,3) represents volume mean diameter; Dv90 represents the diameter wherein 90% of the volume distribution is below this value; Dv50 represents the diameter wherein 50% of the volume distribution is above and below this value. ζ-Potential of oil droplets was determined using a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, Worcestershire, UK). The emulsions were diluted 200 fold using MilliQ water prior to measurements. In this paper, D(4, 3) values are mostly used in the discussion section.

2.4. Creaming stability

The emulsions were visually checked for phase separation and oiling-off or creaming. Also, the amount of creaming was measured by storing them in 6 ml sealed graduated tubes at 4 ± 2 °C for 9 days. In this test, Sudan III dye was used to improve the clarity among separated phases. 0.0025% of Sudan III dye was mixed with flax seed oil for 2.5 h at room temperature using a magnetic stirrer. Instead of flax seed oil, oil-colour mixture was used in making the emulsions. The emulsion stability against creaming was monitored by measuring the volume of the lipid-rich layer on top (V_L) and the volume of total emulsion (V_E) in the tube. Creaming stability in terms of creaming index (%) was obtained using the equation (1),

$$\text{Creaming Index } (\%) = (V_E - V_L/V_E) \times 100 \quad (1)$$

For example, if the creaming index is 100%, there is no phase separation in the emulsions.

2.5. Hydrophobicity of milk proteins

Changes to the hydrophobicity of the milk proteins were measured on the aqueous portion of the sonicated emulsions. Aqueous phase of the emulsions were separated by skimming off the fat at 14,000 rpm for 15 min using Hermle centrifuge (Z306) at room temperature (Pearce & Kinsella, 1978). Before fluorometric assay, the aqueous phase was vortexed for 30 s and diluted with MilliQ water in the ratio of 1:20 in order to prepare the aqueous milk protein solution. The changes to the protein content of the aqueous milk protein solution was monitored by the Bradford Assay according to manufacturer's instructions (Sigma Aldrich Pty Ltd, Sydney, Australia) at 595 nm using UV-VIS Spectrophotometer (Carey 3E, Varian, Palo Alto, CA, USA). A stock solution of 0.008 M 1-anilinonaphthalene-8-sulfonate (ANS) was prepared in 0.1 M pH 7 phosphate buffer. It was wrapped in aluminium foil to prevent light exposure and stored at room temperature.

In the assay, the required amounts of aqueous milk protein solutions were made up with 10 ml of phosphate buffer and 20 μL of ANS solution to obtain a set of diluted samples of aqueous milk protein solution at different dilution factors viz., ∞, 65, 33, 22, 16