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# Ultrasound-assisted preparation of a human milk fat analog emulsion: Understanding factors affecting formation and stability

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ABSTRACT

The aim of this work was to investigate the application of ultrasound in the formation of a stable human milk fat (HMF) analog emulsion with macronutrients (lecithin and protein) as emulsifiers required for infant formula, at low levels and within the ranges of infant formula standards; in addition, the aim was to evaluate the stability of the emulsion to various environmental stresses. Increasing the lecithin concentration and decreasing the HMF analog amount yielded a stable HMF analog emulsion with a smaller mean particle size. Increasing the ultrasonic power and time favored decreasing particle sizes. The emulsion obtained under optimized conditions was stable at a pH of 6.4–8.0, at heating temperatures of 50 °C–90 °C, and with sugar levels and sodium ion strengths that met infant formula standards. Storage below 25 °C for 7 days did not substantially change the physicochemical stability of the emulsion. The results provide information useful for manufacturing high-quality infant formula; ultrasonic emulsification can be used to produce a stable HMF analog emulsion using protein and lecithin at low levels to meet infant formula standards.

## 1. Introduction

Human milk is the exclusive source of energy and biologically active components for the growth and development of breast-fed infants. Human milk is an oil-in-water emulsion containing many components such as whey protein, casein, and milk fat globules. Milk fat globules, a major source of energy in human milk, are composed of a triacylglyceride core enveloped by a trilayer membrane containing proteins, enzymes and lipids (Lopez and Ménard, 2011). Human milk fat (HMF), comprised of 98% triacylglycerides, has a unique stereospecific structure (e.g., oleic-palmitic-oleic or oleic-palmitic-linoleic) of the glycerol backbone (Wang et al., 2009). This unique structure plays a vital role in the uptake of calcium, fat and energy in infants (Innis, 2011). In contrast, there are significant losses of palmitic acid (energy) and calcium if palmitic acid is predominantly esterified at the sn-1,3 positions of fats and oils such as in cow's milk and vegetable oils commonly used in traditional infant formula (Lien, 1994). This is why numerous approaches have been proposed in recent decades to alter the structure of triacylglycerides in fats and oils to mimic HMF as much as possible (Zou et al., 2016). Although the enzymatic synthesis of a HMF analog to mimic HMF has been fully studied, few reports are available on the physical production of an HMF analog from fats and oils. Physical techniques such as dry fractionation to produce an HMF analog have several advantages over enzymatic synthesis methods, including being a relatively low-cost process, with a low energy input required for fractionation.

In addition to the triacylglyceride structure, emulsion structures such as droplet size as well as the nature of droplet interfaces in infant formula and human milk also differ remarkably (Michalski et al., 2005). Factors including the type of surface of emulsion droplets and the manufacturing processes used (i.e., pasteurization, homogenization) could affect *in vitro* digestion profiles of milk fat globules and the pattern of released fatty acids (Bourlieu et al., 2015; de Oliveira et al., 2016; Gallier et al., 2013). These differences in *in vitro* digestion may have potential physiological implications in infants. Therefore, it is necessary to pay attention to the emulsion structure as well as the triacylglyceride structure of the HMF analogs intended for infant nutrition and health. However, few reports are available on the production of a HMF analog emulsion to formulate infant formula powder. The formation and stabilization of a HMF analog emulsion is a necessary and crucial step in manufacturing infant formula. Zou and Akoh (2013) reported that a HMF analog obtained from enzymatic synthesis was formed into an infant formula emulsion using lecithin and monoacylglycerides as emulsifiers as well as locust bean gum and carrageenan as thickeners using a high-pressure homogenizer; the effect of permitted antioxidants on the lipid oxidation of the HMF-based infant

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formula emulsion was examined (Zou and Akoh, 2015). To the best of our knowledge, there are no studies available on the application of other emulsification methods such as ultrasonic emulsification to produce a HMF analog emulsion using only macronutrients required for infant formula as emulsifiers without using additional thickeners. Lecithin and proteins such as whey protein or have been used as emulsifiers to prepare regular O/W emulsions using a high-pressure homogenizer (Chung et al., 2017). However, the production of a HMF analog emulsion is different from the production of regular emulsions, which use other common oils. The main difference is that the levels of protein, lecithin, and HMF that are used to produce a HMF analog emulsion should be within the required ranges of infant formula standards. Otherwise, for preparing regular emulsions, the levels of emulsifiers such as protein can be high enough to achieve a high loading capacity of oil or other lipophilic bioactives with good stability. It is unclear how formulation design influences the formation and stability of a HMF analog emulsion formed by ultrasound-assisted emulsification. Furthermore, it is unclear how environmental stresses influence the physicochemical stability of a HMF analog emulsion formed by ultrasound-assisted emulsification. More efforts need to be made to address these research aspects to provide useful information for producing high-quality infant formula from a HMF analog emulsion.

The objective of this study was to fabricate a stable HMF analog emulsion with two types of macronutrients (proteins and lecithin) as emulsifiers required for infant formula, at levels within ranges of infant formula standards. First, leaf lard was fractionated to obtain a liquid fraction rich in oleic-palmitic-oleic structure, and the liquid fraction was blended with several vegetable oils to produce a HMF analog. Second, the effect of formulation parameters and ultrasonic emulsification conditions on the particle size and stability of the HMF analog emulsion was examined. Furthermore, the influence of environmental stresses on the physicochemical properties of the HMF analog emulsion was evaluated.

## 2. Materials and methods

### 2.1. Materials

Leaf lard was purchased from a local market (Chongqing, China). Camellia seed oil was purchased from Jiangxi Chunyuan Green Food Co., Ltd. (Jiangxi, China). Virgin coconut oil was purchased from Shanghai Xinchen Trade Co., Ltd. (Shanghai, China). Soybean oil was supplied by Chongqing Red Dragonfly Oil Co., Ltd. (Chongqing, China). Lecithin from soybean (CAS No. 8002-43-5), whey protein (CAS No. 9006-59-1) and casein from bovine milk (CAS No. 9000-71-9) were purchased from Hefei Bomei Biotechnology Co., Ltd. (Anhui, China). A standard mixture with 37 fatty acid methyl esters (Supelco® 37 Component FAME Mix) was purchased from Sigma-Aldrich. Ultrapure water was used to prepare all the emulsions and aqueous solutions. All the other reagents and solvents used were of analytical grade.

### 2.2. HMF analog preparation

Leaf lard was fractionated in a water bath (DF-101S mechanical stirrer, Gongyi Yuhua Instrument Co., Ltd., Henan, China) under stirring at 46 rpm. Fractionation temperature was set as follows: 60 °C was set as the initial temperature for 20 min, and then the temperature was decreased to 33 °C at 4 °C/min. The liquid fraction obtained from the fractionation was named 33L-lard and was used as the major ingredient for the HMF analog preparation. The HMF analog was made by blending 33L-lard (54.80% by weight), camellia seed oil (19.78%), soybean oil (15.14%), and virgin coconut oil (10.28%).

### 2.3. HMF analog emulsion preparation by ultrasonic emulsification

The emulsion was prepared by ultrasonic emulsification. Initially, the aqueous phase was prepared by dissolving proteins (whey protein/casein weight ratio = 3:2) and lecithin in water, and the mixture was then stirred at 30 °C for 2 h. The pH of the aqueous phase was adjusted to 6.8 by adding 0.1 mol/L NaOH solution. Sodium azide solution was then added to the aqueous phase to obtain a final concentration of 0.02% to inhibit microbial growth only for experimental observation. The aqueous phase was then stored at 4 °C for 12 h to allow for protein hydration. Second, the HMF analog was blended with the aqueous phase under stirring at 30 °C for 5 min, and a coarse emulsion was made using a high-speed homogenizer (ULTRA-TURRAX T18, IKA, Staufen, Germany) operated at 20,000 rpm for 2 min. Finally, the coarse emulsion (100 g) in a 150 mL-beaker was subjected to ultrasonic emulsification using a sonicator (JY98-IIIDN, 20 kHz, Ningbo Scientz Biotechnology Co., Ltd., Zhengjiang, China). A probe at 2 cm diameter was immersed 4.5 cm below of the liquid surface. The work time and the rest time for sonication were set at 5 s and 3 s, respectively. The temperature of the samples was controlled below 45 °C during the ultrasonic emulsification process by placing the sample in a larger beaker containing ice and water. The resulting emulsion was characterized in terms of the z-average diameter, polydispersity index (PDI), ζ-potential, surface tension, viscosity and turbidity.

The influences of formulation variables and ultrasonic emulsification conditions on the formation and stability of the emulsions were investigated. The influence of lecithin concentration (0.05%–0.50%, based on the weight of the resulting emulsion) was studied under the following conditions: 1.75% protein concentration, 1.75% HMF analog amount, 300 W ultrasonic power, and 15 min ultrasonic time. The influence of the amount of HMF analog (1.75%–3.55%) was examined under the following conditions: 0.50% lecithin concentration, 1.75% protein concentration, 300 W ultrasonic power, and 15 min ultrasonic time. The influence of protein/HMF analog weight ratio (0.49–1.77) was investigated under the following conditions: 0.50% lecithin concentration, 300 W ultrasonic power, and 15 min ultrasonic time. The influence of ultrasonic time (0–35 min) was investigated under the following conditions: 0.50% lecithin concentration, 1.75% protein concentration, 1.75% HMF analog amount, and 300 W ultrasonic power. The influence of ultrasonic power (60–600 W) was investigated under the following conditions: 0.50% lecithin concentration, 1.75% protein concentration, 1.75% HMF analog amount, and 15 min ultrasonic time.

### 2.4. Influence of environmental stresses on the stability of the HMF analog emulsion

The HMF analog emulsion was prepared under optimized conditions of lecithin concentration of 0.50%, protein concentration of 1.75%, HMF analog amount of 1.75%, ultrasonic power of 300 W, and ultrasonic time of 15 min. The influence of environmental stresses on the physical stability of the HMF analog emulsion obtained from the optimized conditions was investigated by determining changes in the z-average diameter, PDI and ζ-potential. The influence of pH was measured by adjusting the pH of the HMF analog emulsions to 6.4–8.0 by adding 1 mol/L HCl or NaOH. The influence of ionic strength was studied by adding NaCl or $CaCl_2$ solution to the HMF analog emulsion. The influence of sugar concentration was studied by adding lactose or glucose to the HMF analog emulsion. The influence of heating temperature was evaluated by keeping the emulsion under stirring conditions at various temperatures (50–90 °C) for 25 min followed by rapid cooling in an ice-water bath to room temperature. The influence of storage conditions (temperature and time) was investigated by keeping the emulsions at 4 °C, 25 °C and 37 °C.

The influence of storage conditions (temperature and time) on the chemical stability of the HMF analog emulsion from optimized conditions was investigated by keeping the emulsion samples at 4 °C, 25 °C and 37 °C. The emulsion samples were withdrawn at different time intervals to determine peroxide value. Peroxide value was used to