Preparation and Characterization of Ergocalciferol-Loaded Nanodispersions Stabilized by Different Emulsifiers

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Abstract of thesis

Vitamin D is one of the essential bioactive compounds for human being, due to its special health-promoting functionality. Previous studies have demonstrated that this substance contributes to the development of bone, teeth and cartilage. Moreover, it also prevents cancer, and enhances the heart and immune system. Ergocalciferol is a type of plant-based vitamin D, which is naturally present in a low amount in wild mushrooms, whereas another type called cholecalciferol can be produced in the human skin via the exposure of sunlight. However, there is still an estimated one billion people worldwide who either have vitamin D deficiency or insufficiency due to limited sun exposure, extensive UV-protecting sun cream usage, or poor dietary intake. For these above-mentioned reasons, fortified food and beverage products with vitamin D are gaining attention in food industry nowadays. However, vitamin D has poor water-solubility, chemical instability towards environmental stresses and variable oral bioavailability, which strongly limit the application of this vitamin as a functional ingredient to be incorporated into aqueous-based food products. Encapsulation of vitamin D in nanodispersion-based delivery systems could be an attractive strategy to overcome these limitations. Selection of an appropriate emulsifier is extremely important to prepare a stable and efficient nanodispersion. The author therefore aimed to formulate and characterize ergocalciferol-loaded nanodispersions stabilized by different emulsifiers.

Firstly, the author prepared ergocalciferol-loaded nanoemulsions by using high-pressure homogenization method. The effects of emulsifier type and concentration, oil type and concentration, and homogenization pressure on the droplet characteristics of nanoemulsions produced by high-pressure homogenizer were investigated. The results showed that the average size of emulsified droplets decreased with increasing operating pressure and emulsifier concentration. Nano-sized droplets ($d_{4,3}$ < 150 nm) could be successfully formed using soybean oil, perilla oil and medium chain triglyceride (MCT). The nanoemulsions stabilized by modified lecithin (ML), sodium caseinate (SC) or decaglycerol monooleate (MO7S) showed similar droplet size and size distribution.

Secondly, the author investigated the effect of emulsifier type on the stability of ergocalciferol-loaded nanoemulsions. The stability of resulting nanoemulsions was evaluated when they exposed to different environmental stresses and during 30 days of storage at 25 and 55 ^{o}C. Results showed that the emulsions prepared by MO-7S or ML were stable against a wide range of pH (2-8), while SC-stabilized emulsions showed instability with extensive droplet aggregation at pH 4 and 5. Only ML-stabilized emulsions showed droplet growth due to coalescence when treated at high NaCl concentration (300-500 mM). In the absence of glucose, SC-stabilized O/W emulsions showed better freeze-thaw stability, in comparison to those formed with ML or MO-7S emulsifiers. The emulsion produced by ML was found to be stable to droplet aggregation at high temperatures (80-120 ^{o}C) for 1 h. All the O/W emulsions stored at 25 ^{o}C showed good physical and chemical stabilities. However, the chemical stability of ergocalciferol in emulsion system decreased in order of ML > MO-7S >> SC during storage at 55 ^{o}C for a period of 30 days.

Thirdly, the author examined the effect of emulsifier type on the *in vitro* bioaccessibility of ergocalciferol-loaded nanoemulsions (mouth, stomach and small intestinal phases). Results indicated that the droplet size, size distribution, ζ-potential and microstructure of nanoemulsions during digestion depended on the emulsifier type. The fate of lipid in the small intestinal phase also relied on the emulsifier type, with the free fatty acids release rate decreasing in the following order: MO7S > ML-MO7S > ML > SC. The bioaccessibility of ergocalciferol in nanoemulsions prepared using MO7S, ML, and ML-MO7S was 62%, 64%, 65%, respectively, which was higher than that stabilized by SC, 12%. No significant loss of ergocalciferol was observed in all nanoemulsions after full digestion; they were chemically stable against digestion conditions, regardless of the emulsifier type.

Lastly, the author investigated the formulation, stability and bioaccessibility of ergocalciferol nanodispersions stabilized by ML and SC as natural emulsifiers. The mean particle size of nanodispersions stabilized by ML, 56 nm, was much smaller than those stabilized by SC, 112 nm. The ML-stabilized nanodispersions were stable over a wide range of pH, NaCl concentrations and heating, but became unstable with slight increase in particle size when exposed to $CaCl_2$ solution. In comparison, SC-stabilized nanodispersions were relatively unstable, becoming aggregation under the conditions of pH 4-5, $CaCl_2$ addition and heating. Long-term stability for ergocalciferol were observed in both ML- and SC-stabilized nanodispersions. The bioaccessibility of ergocalciferol was strongly dependent on the emulsifier type, with ML providing much higher bioaccessibility than SC.

The author has found that modified lecithin is a promising natural emulsifier for preparing ergocalciferol nanoemulsions/nanodispersions with excellent stability and ergocalciferol bioaccessibility. Comparing with nanoemulsions, ergocalciferol nanodispersions could be prepared using much lower level of emulsifier, which seems to be more practical in food production.

Abstract of assessment result

【Review】

Vitamin D deficiency is prevalent in many populations, which leads to adverse health effects. Thus, it is important to develop foods and beverages with this water-insoluble micronutrient. This work therefore aimed at developing effective dispersion systems for delivering ergocalciferol (vitamin D_2). In order to produce O/W nanoemulsions with relatively small droplets, the author investigated and optimized the operating parameters of high-pressure homogenization method. The factors influencing the physiochemical stability of the resulting nanoemulsions were then systematically investigated, and the corresponding mechanisms were well clarified. In addition, the author elucidated the relationship between emulsifier type and ergocalciferol bioaccessibility of nanoemulsions via *in vitro* digestion model. Lastly, the author clarified that effective ergocalciferol nanodispersions with high stability and bioaccessibility could successfully formulated via solvent displacement method using natural emulsifiers such as enzymatically modified lecithin. A variety of food products (e.g., soups and beverages) could be fortified with ergocalciferol-enriched nanoemulsions/nanodispersions, which may solve vitamin D deficiency prevalent in many people. Overall, the results obtained by the author in this study would contribute to a food innovation through the development and efficient design of nano-delivery systems for vitamin D.

【Result】

The final examination committee conducted a meeting as a final examination on 18 June, 2018. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Food Innovation of Life Science Innovation Program.