JOURNAL OF NUTRITION FASTING AND HEALTH

Co-encapsulation of Vitamin D and Calcium for Food Fortification

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ARTICLEINFO

Article type:
Research Paper

Article History:
Received: 23 Aug 2019
Accepted: 08 Dec 2019
Published: 20 Dec 2019

Keywords:
Modified Starch
Ultrasound
Homogenization
Encapsulation Efficiency

ABSTRACT

Introduction: Receiving adequate vitamin D and calcium is essential to bone health and reducing the risk of osteopenia and osteoporosis. The present study aimed to co-encapsulate vitamin D and calcium using the ultrasonic emulsification technique and assess the physicochemical properties of the obtained emulsions and capsules.

Methods: Two types of calcium salts and vitamin D_3 were used as the core materials in modified starch microcapsules. The effects of the sonication process and formulation on the physicochemical properties of the emulsions and dried powder of the final spray were investigated.

Results: Use of ultrasound homogenization with 80% maximum power for three minutes provided the optimum process conditions to fabricate the emulsions. In total, 16 emulsion formulations were prepared, and their physicochemical properties were evaluated. All the emulsions had narrow size distribution with more than 79% encapsulation efficiency. Based on the particle size and encapsulation efficiency, two formulations were selected and further characterized. Calcium carbonate and stearate containing the microcapsules had more than 89% encapsulation efficiency with the particle size of 2 and 2.5 micrometers, respectively. In addition, 70% of vitamin D was preserved in the encapsulated form during eight month.

Conclusion: According to the results, the co-encapsulated microcapsules of vitamin D_3 and calcium could be used to prolong the stability and improve the solubility of vitamin D_3 for food applications.

► *Please cite this paper as*:
Bostan A, Ghaitaranpour A. Co-encapsulation of Vitamin D and Calcium for Food Fortification. J Nutrition Fasting Health. 2019; 7(4): 229-234 DOI: 10.22038/jnfh.2019.42733.1216

Introduction

The role of vitamin D in mineral balance and skeletal maintenance has been extensively reported in the literature (Lee et al., 2008). Vitamin D deficiency is recognized as a pandemic micronutrient malnutrition disorder (Harinarayan et al., 2009; Kumssa et al., 2015), which is associated with the increased risk of osteoporosis, rickets, calcium-phosphorus imbalance, parathyroid imbalance, hypertension, and diabetes. Several studies have suggested that rickets and osteoporosis are caused by both vitamin D and calcium deficiency. As a result, sufficient intake of vitamin D and calcium simultaneously guarantees human health.

Food fortification is acknowledged as the most cost-effective health intervention available to address micronutrient malnutrition (Allen et al., 2006). However, the fortification of vitamin D has been a challenge for the food industry due to its instability and heterogeneous distribution in the food matrix (Maurya and Aggarwal, 2017; Tsiara and Weinstock, 2011). The poor water-soluble nature of vitamin D does not allow its incorporation into beverages or aqueous foods (Guttoff et al., 2015; Hasanvand et al., 2015). Furthermore, the lipophilic nature of this vitamin makes it sensitive to ultraviolet light, oxidation, and processing conditions. On the other hand, the addition of calcium to some food products (e.g., beverages) may cause turbidity and off tastes. In such cases, encapsulation could be used to prevent organoleptic disorders.

Encapsulation techniques protect bioactive ingredients against hostile conditions and increase their solubility in aqueous systems. Furthermore, encapsulation facilitates the controlled release and administration of optimum doses, thereby helping avoid hypervitaminosis syndrome and the associated complications.

In a study, Winuprasith et al. (2018) encapsulated vitamin D_3 in oil in water Pickering emulsions and assessed its bioaccessibility. In addition, Delavari et al. (2015) designed an alpha-lactalbumin delivery system to solubilize and transport vitamin D_3

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within food systems. In another research, Hassanvand et al. (2015) developed starch-based nanoparticles for the entrapment of vitamin D_3 and investigated their potential for milk fortification.

To the best of our knowledge, no previous studies have been focused on the co-encapsulation of calcium and vitamin D. The present study aimed to co-encapsulate vitamin D_3 and calcium using the ultrasonic emulsification technique and assess the physicochemical properties of the emulsions and capsules. Our findings provide important implications for the utilization of functional foods.

Materials and Methods

Octenyl succinic anhydride (OSA)-modified starch (HICAP100), which is a modified food starch derived from waxy maize, was obtained from the National Starch and Chemical Limited (UK), and vitamin D_3 (VD_3) was purchased from Zahravi Pharmaceutical Co., Iran. HPLC grade VD_3, span 80, and tween 80 were also obtained from Sigma-Aldrich, Shanghai Trading Co. Ltd. (Shanghai, China). All the other chemicals were of the analytical grade.

1-Capsules Preparation

1-1-Preparation of Emulsions

Aqueous solutions were prepared by dissolving the OSA-modified starches (20% w/w) in distilled water at the temperature of 50 °C under gentle stirring for 30 minutes in order to enhance hydration, followed by cooling to room temperature. Exact quantities of VD_3, calcium stearate or carbonate were weighted and dissolved in ethanol through sonication. Afterwards, the organic phase was added to the aqueous solution drop-wise with the agitation of a rotor-stator homogenizer (model: Ultra-Turrax IKA T18 Basic, Wilmington, NC, USA), which was operated at 5,000 rpm for five minutes at room temperature (Lesmes et al., 2008). Finally, fine emulsions were produced using an ultrasound-assisted homogenizer (model: HD 3200, Bandelin, Germany).

1-2-Optimization of Sonication Parameters

Pre-emulsions were subjected to high-intensity sonication at the operating frequency of 20 kHz using an ultrasonic processor (750 W; Sonics, USA), which was equipped with a 19-millimeter diameter probe. The applied power was 40%, 60%, 80%, and 100% of the maximal equipment power at the sonication times of 1.5, three, and 4.5 minutes.

1-3-Spray-drying Process

The homogenized emulsions were dried in a spray dryer (model: B190, BUCHI, Germany), which was equipped with a co-current nozzle atomizer. The inlet and outlet temperatures were 180 °C and 60 °C, respectively with the feed rate of 10 ml/min. The obtained nanocapsules were sealed into plastic bag and preserved at the temperature of -18 °C for further analysis.

1-4-Formulation Development

Based on preliminary tests, various concentration of emulsifiers (span 80, tween 80), ethanol, and calcium sources (stearate calcium and carbonate calcium) were used for the formulation of the capsules. In addition, the concentrations of starch and VD_3 were fixed in all the formulations. The developed formulations are presented in Table 1.

Table 1. Formulation, particle size, PDI, EE and Turbidity of D_3 and calcium loaded emulsion

Formulation ingredient / Formulation no.	Tween 80 (%)	Span 80 (%)	Ethanol (%)	Starch (%)	Calcium stearate (%)	Calcium carbonate (%)	VD3 (%)	Particle size (nm)	PDI	EE (%)	Turbidity
1	5	0	5	10	0	0.7	0.2	430	0.23	90	1.785
2	2.5	0	5	10	0	0.7	0.2	536	0.21	89	1.843
3	5	0.5	5	10	0	0.7	0.2	730	0.30	84	1.902
4	2.5	0.5	5	10	0	0.7	0.2	901	0.28	89	2.015
5	5	0	10	10	0	0.7	0.2	890	0.21	79	2.010
6	2.5	0	10	10	0	0.7	0.2	950	0.33	81	2.222
7	5	0.5	10	10	0	0.7	0.2	1130	0.19	85	2.274
8	2.5	0.5	10	10	0	0.7	0.2	1100	0.21	87	2.301
9	5	0	5	10	0.3	0	0.2	650	0.23	89	1.634
10	2.5	0	5	10	0.3	0	0.2	740	0.19	89	1.710
11	5	0.5	5	10	0.3	0	0.2	892	0.20	81	1.698
12	2.5	0.5	5	10	0.3	0	0.2	956	0.21	84	1.723

13	5	0	10	10	0.3	0	0.2	908	0.31	87	1.793
14	2.5	0	10	10	0.3	0	0.2	1035	0.27	85	1.903
15	5	0.5	10	10	0.3	0	0.2	1100	0.31	84	1.876
16	2.5	0.5	10	10	0.3	0	0.2	1008	0.19	83	1.900

2-Physicochemical Characterization

2-1-Particle Size and Polydispersity Index

The mean particle size and polydispersity index (PDI) of the emulsions and loaded particles were determined using dynamic light scattering (DLS; Zetasizer Nano ZS, Malvern Instruments, UK). PDI was used to measure the size distribution of the particles, and lower PDI was associated with lower size distribution. All the measurements were performed in triplicate for each sample.

2-2-Encapsulation Efficiency (EE)

Encapsulation efficiency (EE) showed the fraction of the encapsulated material that was incorporated into the capsules compared to the total amount of the core material that was initially added (Eq. 1). The calibration curve of VD_3 in hexane (concentration in terms of absorption) was plotted, and the obtained data were transformed in the VD_3 weight. Following that, 10 milligrams of the spray-dried particle powder was washed with five milliliters of n-hexane, and the suspension was filtrated through a Whatman No. 1 filter paper. Afterwards, the filtrate was subjected to spectrophotometric measurements at the wavelength of 264 nanometers using a UV/Vis spectrophotometer (model: UV-160A, Shimadzu, Japan). The procedure was repeated until the absorption of the last filtrate became zero. All the filtrates were combined, and the absorbance was measured to determine the free VD_3. The remaining nanoparticles after filtration were dried and weighed (Hasanvand et al., 2015).

$$EE\% = 100 - \left(\frac{weight\ of\ free\ vitamin\ D}{weight\ of\ initial\ vitamin\ D\ used} \times 100\right)$$

2-3-Turbidity

The turbidity of the emulsions was measured using a UV/Vis spectrophotometer (model: UV-160A, Shimadzu, Japan) at the wavelength of 520 nanometers.

4-Color, Moisture Content, and Density

The color attributes (Hunter L*, a* and b* values), moisture content, and density of the selected formulations (spray-dried powder) were investigated (Karaca et al., 2013).

5-Vitamin D_3 Stability

The selected capsule powder was stored in sealed glass vials at the temperature of 25°C for eight months, and the changes in the VD_3 content were monitored at 30-day intervals.

6-Statistical Analysis

All the experiments were performed in triplicate, and the mean values were reported. Data analysis was performed in SPSS version 16 using the analysis of variance (ANOVA), and the results were compared using Duncan test at the significance level of 5%.

Results and Discussion

Effects of Sonication Parameters

Determining the optimum power and time for the ultrasound-assisted process was essential to reaching particles with small and narrow distribution and minimizing the energy loss and production costs. Figure 1 shows the emulsion droplet size for the applied power and times. As can be seen, the emulsion droplet size significantly decreased with the increased sonication power from 40% to 100% of the maximum power in all the applied time intervals. In another research, Jafari et al. (2007) reported that sonication time had a significant effect on particle size. On the other hand, increased sonication time from 1.5 to three minutes resulted in decreased droplet size at all the applied powers. This could be due to the increased energy density with sonication time, which caused more droplet deformation and disruption, thereby leading to smaller particles. Further sonication time (up to 4.5 minutes) also resulted in the increased size at all the applied powers, with the exception of 40% power, suggesting that aggregation would occur at prolonged processes. Consequently, sonication intensity and time of 80% and three minutes were selected for emulsion production.

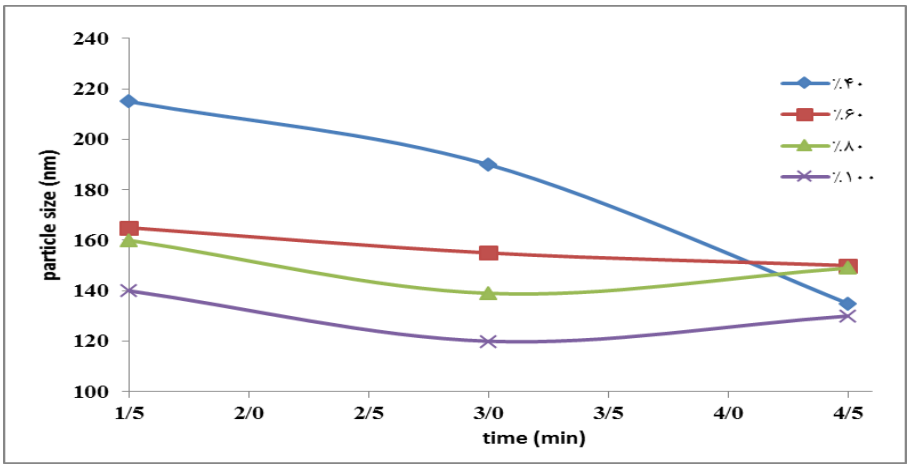

Figure 1. The effect of sonication time and intensity on emulsion droplet size.

Effect of Formulation Ingredient

In the present study, various formulations were prepared under optimized sonication conditions. The results of emulsion turbidity, particle size, PDI, and EE are shown in Table 1. Accordingly, the particle size was within the range of 430-1,130 nanometers, and increased emulsifier concentration resulted in the significant reduction of the particle size. A similar trend has also been reported in the other studies in this regard (Abbas et al., 2014). This could be due the fact that the larger surface area of droplets could be covered when sufficient concentrations of the emulsifier are available during homogenization, which in turn provides stability to the newly-formed droplets.

According to the findings of the current research, the carbonate calcium containing the formulations had a smaller size compared to the emulsions containing calcium stearate (Table 1). Moreover, the size and solubility of the encapsulant largely influenced the particle size of the emulsion droplets (Sun-Waterhouse et al., 2012). Encapsulation efficiency is an indicator of the ability to entrap core material and is generally affected by the fabrication method and ingredients used in the preparation of the emulsion (Kiani et al., 2017; Ozturk et al., 2015). In the present study, the encapsulation efficiency of the samples was within the range of 79-90%, which indicated the high amount of encapsulated VD_3 (Table 1).

In general, EE in the capsules containing the calcium carbonate was observed to be higher compared to the calcium stearate containing the capsules in the current research. In addition, the obtained encapsulation efficiency was superior to the zein-based particles with (52.2%) or without carboxymethyl chitosan coating (71.5%) (Luo et al., 2012). In the present study, the alginate-based nanoparticles with the hydrophobic core were used to encapsulate VD_3, and the loading efficiency was determined to be 45.8±1.55-67.6±2.76% (Li et al., 2011).

PDI is a measure of the distribution of particles/droplets, which was estimated to be within the range of 0-1 in the current research. The PDI value of one indicated that the emulsion was highly polydispersed. According to our findings, all the formulations had PDI values of less than 0.33, which suggested the narrow size distribution of the emulsion droplets. Furthermore, transparency could be considered an indirect indicator of small-sized, homogenous, and stable emulsion. According to the information in Table 1, the emulsions containing calcium stearate were less turbid compared to the emulsions containing calcium carbonate.

Selection of the Most Suitable Formulation

In order to select the most suitable formulations, the capsules with the smallest size, highest EE, and lowest turbidity in the emulsions with both calcium stearate and carbonate were selected for further analysis. The obtained results indicated that formulations number nine and one were most suitable for calcium stearate and carbonate, respectively.

Characterization of the Selected Formulations

The spray-dried microcapsules of the selected formulations are depicted in Figure 2. Table 2 shows the Hunter color parameter L*, a* and b*, moisture content, and density of the capsule

powder. The obtained resulted demonstrated no significant difference in the L* values between the capsules containing calcium stearate/carbonate, while the a* and b* values were higher in the capsules containing calcium stearate. The moisture content of the powder was estimated at 3.4% and 3.7%, which was within the maximum moisture specification for dried powder in the food industry (3-4%) (Klinkesorn et al., 2006). In addition, the density of the calcium carbonate and calcium stearate capsule powders was calculated to be 0.23 and 0.17 g/cm^3, respectively.

Table 2. Properties of selected formulations spray dried powder.

	L*	a*	b*	Particle size (µm)	EE (%)	Moisture content (%)	Density (gr/cm^3)
VD_3 & Ca. carbonate	97	3	0.2	2	90	3.7	0.23
VD_3 & Ca. stearate	98	2.2	0.5	2.5	89	3.4	0.17

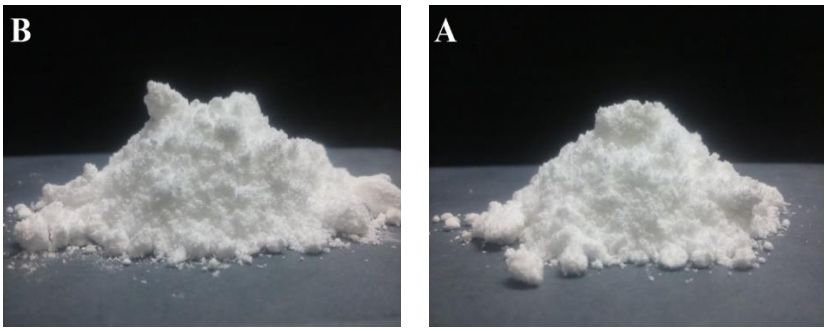

Figure 2. Spray dried powder of vitamin D_3 and calcium stearate (A) and vitamin D3 and calcium carbonate (B) microcapsules.

VD_3 Stability

Based on the smaller size and higher EE and calcium content, formulation number one was selected for stability assessment in terms of VD_3 retention at the temperature of 25 °C and relative humidity (RH) of 70% during eight months. As is depicted in Figure 3, more than 70% of the VD_3 was preserved in the capsules, while free VD_3 disappeared within two months. Therefore, it could be concluded that encapsulation successfully prolonged the shelf life of VD_3. In fact, encapsulation provoked the immobilization of the vitamin, which in turn reduced its reactivity and access of oxidizing agents (Ron et al., 2010).

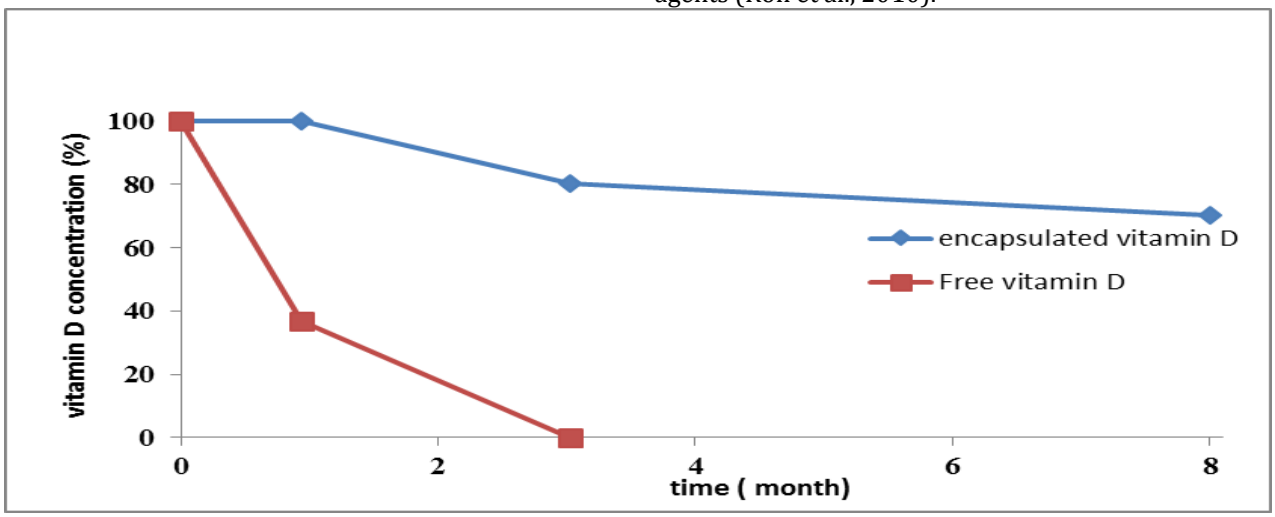

Figure 3. Stability of encapsulated vitamin D_3 in 25°C during 8 month.

Conclusion

According to the results, VD_3- and calcium-loaded microparticles were successfully developed using OSA-modified starch. Therefore, it could be inferred that applying ultrasound homogenization at 80% maximum power for three minutes provided the optimum process condition to fabricate the emulsions. All the emulsions had narrow size distribution and more than 79% encapsulation efficiency. Furthermore, the spray-dried powder of the selected formulations had the particle size of two and 2.5 micrometers and 90% and 89% encapsulation efficiency in the microcapsules containing VD_3 and calcium carbonate and calcium stearate, respectively.

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