



Review

Encapsulating Vitamin D: A Feasible and Promising Approach to Combat Its Deficiency

Mina Esmaeili^{1,2}, Reza Yekta^{3,2}, Abdol-Samad Abedi^{4,2}, Kiandokht Ghanati^{2,5}, Robabe Zamani Derav⁶, Anahita Houshyarrad^{1,2*}, Zeinab Saberi Dehkordi⁷, Marjan Ajami^{8,2*}, Maryam Mahmoudzadeh^{9,10,11}

¹Department of Nutrition Research, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

²National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

³Student Research Committee, Department of Food Science and Technology, Faculty of Nutrition and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

⁴Department of Research Deputy, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

⁵Research and Education Affairs Office of International Affairs, Food Safety Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

⁶Hepatology and Nutrition Research Center, Research Institute for Children's Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

⁷Department of Chemistry, Isfahan University of Technology, Isfahan, Iran.

⁸Department of Food and Nutrition Policy and Planning Research, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

⁹Drug Applied Research Center and Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

¹⁰Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

¹¹Department of Food Science and Technology, Faculty of Nutrition and Food Science, Tabriz University of Medical Sciences, Tabriz, Iran.

Article Info

Article History:

Received: 10 February 2021

Accepted: 29 July 2021

ePublished: 4 August 2022

Keywords:

-Bioavailability
-Encapsulation
-Enrichment
-Food
-Fortification
-Vitamin D

Abstract

Vitamin D (VD) deficiency is a significant issue affecting a large population around the world. As its natural sources are limited, people must constantly fortify their VD. Encapsulating VD increases its bioavailability and stability during processing and storage; hence it has promising potential to avoid VD deficiency. This study reviews current methods of VD fortification and encapsulation. Two predominant methods of VD fortification, i.e., biofortification and direct fortification, are advantageous over VD supplementation. However, significant VD losses occur during processing, storage, and passing across the stomach which can be minimized through encapsulation methods, i.e., micro and nanoencapsulation. Moreover, the capsule features like size, wall-to-core ratio, wall material, carrier oil composition, and encapsulation technique significantly affect VD bioavailability. To assess the optimum encapsulation procedures and possible risks in food fortification, comprehensive in vitro and in vivo studies must be conducted. Depending on the staple food products of a specific region, both VD fortification strategies have great potential in different countries. Besides, the risk of VD overdose due to fortifying a single staple food product is higher than fortifying various staple food products.

Introduction

Vitamin D (VD) is a lipid-soluble vitamin comprised of ergocalciferol (VD₂) and cholecalciferol (VD₃). VD₂ is naturally synthesized in plant resources such as fungi and yeasts, whereas VD₃ is produced in vertebrates and lichens.¹ For a long time, VD deficiency or insufficiency has been a global problem from which many of the world population is suffering.² Daily exposure to sunlight can provide up to 80% of the body's VD requirement.³ However, sun-induced VD synthesis in the body is affected by the following circumstances: geographical latitude of people's residence, wearing sunscreens, skin pigmentation, season, and clothing type.⁴ VD deficiency causes rickets

(mostly in children) and osteoporosis (in adults).⁵ Also, prolonged VD insufficiency is associated with several non-musculoskeletal disorders, including certain types of cancers,^{6,7} Parkinson's disease,⁸ multiple sclerosis,⁹ cardiovascular disease,¹⁰ diabetes type 2,¹¹ and immune system dysfunction.¹²

Although there is no unanimous agreement among scientists regarding the cut-off point for VD deficiency, concentrations lower than 25 nmol/L serum 25-hydroxyvitamin D [25(OH)D] are considered deficient. Furthermore, in some countries, 50 and 75 nmol/L serum 25(OH)D are recommended as the goal and optimal VD levels, respectively, which equate to 600 to 1000 IU VD₃

*Corresponding Author: Marjan Ajami, E-mail: marjan.ajami80@gmail.com & Anahita Houshyarrad, E-mail: anahrad@yahoo.com

©2021 The Author(s). This is an open access article and applies the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>). Non-commercial uses of the work are permitted, provided the original work is properly cited.

daily intake.¹³ To provide sufficient VD amounts to maintain health, taking VD supplements or eating foods containing high amounts of VD is recommended. However, foods such as egg yolk, milk, some oily fish, and meat that are rich in VD are limited.¹⁴ Most recent studies on enriching and fortifying milk, yogurt, cheese, bread, eggs, fruit juice, and meat with VD reported promising outcomes. Therefore, it is recommended to enrich and fortify foods with VD. Fortification is usually done through two procedures: 1) biofortification, which entails DV fortification in the final product through adding its precursor into animal feed, or 2) natural fortification, i.e., directly adding VD into food items.¹⁵

Notwithstanding, the effectiveness of food fortification for VD-enriched foods might be subject to some degrees of VD loss during processing, storage, and even some preparation practices at home, including cooking, boiling, and frying.¹⁶ Also, most vitamin D loss generally happens in the gastrointestinal tract due to VD's susceptibility to acidic conditions.¹⁷ Hence, almost three-quarters of ingested VD is lost.⁵

Encapsulation is a promising technique to preserve sensitive substances such as vitamins, enzymes, antioxidants, flavors, and pigments against detrimental situations, including high temperature, presence of oxygen, and low pH value.¹⁸ In this technique, some materials (known as the wall) coat the sensitive substances (known as the core) to avoid their loss. Based on capsule size, encapsulation can be categorized into microencapsulation and nanoencapsulation with certain specifications and applications for each. Thus, in this article, we aimed to review current VD fortification methods and discuss recent studies on VD encapsulation as far as bioavailability.

Fortification

Fortification entails incorporating non-nutrient or nutrients bioactive compounds into food products that can be used as a public health monitoring measure to not only elevate and treat nutrient deficiencies but also to ensure health in the population.

Fortification through food

Although VD supplementation is a helpful alternative to getting VD through ample exposure to sunlight, fortifying is a promising alternative to solve VD insufficiency in food products as a public health approach that would be beneficial and provide a minimum supply of this vitamin. However, fortifying only a limited number of food products, like milk or bread, may not be enough to meet a community's VD requirements as vitamin content is very variable, especially after fortification.¹⁹ Besides, in any population, some people may suffer from certain disorders such as lactose intolerance, celiac, and other food-related allergies.^{20,21} Thus, a wide variety of food products must be fortified or enriched with VD, and many studies worked on the direct fortification of food products with VD (Table 1) Consuming either 25000 IU VD₂/240 ml whole milk or

25000 IU VD₂/240 ml skim milk and toast containing 25000 IU VD₂²² showed that milk's fat content did not influence the bioavailability of VD in peak serum VD₂ contents. However, when VD₂ was ingested through a food matrix (processed cheese) than through water, its bioavailability increased.²³ To our knowledge, VD did not exist in the UF-permeate serum, which indicated that it might bind with milk proteins, mainly hydrophobic sites of casein micelles.²⁴

To compare the bioavailability of VD among two groups of younger and older adults, consuming processed cheeses containing 600 IU VD₃ on a daily basis for two months caused no significant change in the concentration of serum 25(OH)D, osteocalcin, and parathyroid hormone (PTH)²³ possibly due to inadequacy in this level of fortification when there was negligible exposure to sunlight. Furthermore, both examined populations showed nearly the same VD bioavailability.²³

Besides, supplementing at least 600 IU of VD₃ is recommended on a daily basis.²⁵ Similarly, it is indicated that daily consumption of 200 ml milk containing 600 and 1000 IU VD for three months led to 137.97 (57.18 ± 16.88 nmol/L) and 177.29 % (69.18 ± 21.18 nmol/L) increase in 25(OH)D in comparison with baseline.²⁶ It is worth noting that the percentage of subjects who had VD deficiency before and after this diet was 92.3 and 5.9, respectively, which indicated that the latter concentrations of fortification would effectively meet the population's VD requirement [25(OH)D > 50 nmol/L]. Comparing the efficiency of fortifying 10 µg VD₃/100g wheat flour to that of 0.25 to 7 µg/100 L milk showed that wheat flour fortification was more effective than milk fortification at the concentrations above without the risk of crossing the upper intake level.²⁷ It might possibly be due to the consumption pattern of the population (UK) in using flour in higher proportion than milk.

In Finland, VD₃ food fortification of from 2000 to 2011 increased 25(OH)D levels in adults from 48 to 65 nmol/L.²⁸ Consuming VD sources according to the nutritional recommendations would lead to serum 25(OH)D status higher than 50 nmol/L, which is adequate; hence no supplementation was generally required.²⁸ This improvement in serum 25(OH)D level was mostly ascribed to the ingestion of fluid milk. In contrast, to evaluate the best food carrier of VD in seven low- and middle-income countries, wheat flour, milk, plant oils, and maize flour were fortified with VD where wheat flour containing 1.4 and 2.8 µg VD/100 g flour was the most suitable food carrier to convey VD to the goal population possibly due to the consumption at a higher proportion.²⁹ It is noteworthy that VD₃ had a higher bioavailability than VD₂ thus, fortifying foods with VD₃ would lead to a higher level of serum 25(OH)D.³⁰ However, no studies reported off-flavor due to fortifying various food products with VD. In summary, fortifying staple foods of an area with VD₃ could be considered an effective approach to fighting against VD deficiency in the populations.

Table 1. Fortification of VD in various food products

Food product	Amount of fortification	Main findings	Ref.
Cheddar cheese	VD ₃ : 400 IU/L in three forms: 1) Liposome 2) Homogenized in cream 3) Vitex-D	The recovery of VD in cheese was considerably higher in liposomes (61.5 ± 5.4%) than for VD homogenized in cream (40.5 ± 2.2%) and for Vitex D (42.7 ± 1.7%). After 5 months of storage, a remarkable decline in VD of treatments was initiated, especially liposome-containing one.	31
Cheddar cheese Low-fat cheeses	100 IU VD ₃ /g cheese	The recovery of VD was 91 and 55% in cheddar and low-fat cheeses, respectively, compared to VD fortified milk. VD did not decompose both during processing and after 1 year storage at 3 to 8°C. The chemical composition, yield and flavor of the cheddar cheese were not affected as a result of VD addition. Daily consumption of 4000 IU VD ₃ through cheeses for 2 month showed a remarkable increase in bioavailability of VD compared to control (consumption of not fortified cheese).	32,33
Cheddar cheese	200 or 400 IU VD ₃ /serving	The recovery of VD in the emulsion (90%) form was high than the powder form (about 80%). After 9 months of storage, no loss of VD was observed in cheeses. Homogenization process did not significantly affected the recovery of VD in cheeses. The fortification of VD did not affected sensory properties.	34
Cheddar cheese-like matrix Yogurt Ice cream	50 to 100 VD ₃ IU/g	After 3 months of storage, no loss of VD was observed in yogurt and ice cream. Even though the stability of emulsified VD was higher than pre-dissolved crystalline one in cheese over 3 months of storage, the stability was the same for the two other products. 7 to 9% VD of cheese lost by transferring into whey.	35
HTST processed 2% milk UHT-processed 2% fat chocolate milk Low-fat strawberry yogurt	250 IU VD ₃ /serving	250 IU VD ₃ /serving did not degrade during the shelf lives of the products. 250 IU VD ₃ /serving caused no significant effects on sensory properties	36
Milk	11.7 µg/L VD ₃ + 225 mg/L calcium	Reduction the seasonal decrementation of serum 25(OH)D by more than 50% during winter (54 ± 25 nmol/L in control compared to 62 ± 26 nmol/L in fortified treatment).	37
Milk	600 to 800 IU VD ₃ /200 mL	Daily consumption of 600 and 800 IU fortified milk resulted in 137.97 and 177.29 % increase in serum 25(OH)D, respectively, compared to baseline.	26
Orange juice	1000 IU VD ₃ /240mL	The concentration of serum 25(OH)D increased by about 150% after 12 weeks of daily consumption of orange juice. Concentrations of serum parathyroid hormone (PTH) declined by 25% in comparison with baseline as a result of fortified orange juice ingestion. A seasonal increment of about 45% in 25(OH)D was observed in control subjects as well as no alteration in serum PTH concentration.	22
Orange juice	1000 IU VD ₃ /day 1000 IU VD ₂ /day	After 11 weeks of orange juice consumption, the results indicated the equal bioavailability of VD ₃ and VD ₂ .	38
Pasteurized process cheese	VD ₃ : 100 IU/serving	VD content did not decline in the following stages: production of cheeses, storage at ambient temperature, and refrigeration. Fortification of VD did not cause any off-flavors in cheeses. By heating cheeses to 232°C for 5 min, 25 to 30% of VD lost.	39

Fortification through feed (biofortification)

Although direct fortification of food products is an efficient procedure to transport VD, there are limited food products that can be commercially fortified. Biofortification is a promising approach to broaden the number of food products that can be fortified (see Table 2).

Investigating the effects of different feed diets containing VD₃, mixtures of VD₃ and 25(OH)D, and 25(OH)D alone on the 25(OH)D and VD concentrations of pig's liver, plasma,

and meat indicated that feed diets containing only 25(OH)D did not improve the contents of 25(OH)D and VD. In contrast, substantial increases of the latter components were observed as the level of VD₃ increased in the diet. Furthermore, if 25(OH)D was added as complementary (at lower ratio) to VD₃ in the pigs' diet, the 25(OH)D₃ concentration of serum, liver, and meat could be equally affected by the concentration of both components.⁴⁰

Lots of discrepancies are among studies on VD₃

Table 2. Biofortification of VD in various food products

Food product	Amount of feed fortification	Period of feed fortification	Main findings	Ref.
Chicken egg	VD ₃ fortification: 1) 26.6 µg/kg feed 2) 62.4 µg/kg feed 3) 216 µg/kg feed	6 weeks	Elevating the dose of VD ₃ fortification from 62.4 to 216 µg/kg feed resulted in about 7 times increase in VD of eggs.	41
Chicken egg	1) Regular feed: 1720 or 4280 IU D ₃ /kg 2) Fortified feed: 11200 or 12000 IU D ₃ /kg	Two feeding programs: 1) 30 days 2) 168 days	The highest contents of VD ₃ in egg yolk (about 30 µg/100 g) were reached 8 to 13 days from commencing the diet with the highest dose of vitamin. The VD ₃ content gradually declined to about 22 µg/100 g after feeding with the diet containing the highest dose of vitamin. The high VD ₃ diet did not impair sensory properties or eggshell strength. The diets was not toxic for hens.	42
Chicken egg	VD ₂ : 6000 and 15000 IU/kg feed, VD ₃ : 6000 and 15000 IU/kg feed Regular diet (control): 2,500 IU VD ₃ /kg feed	67 weeks	VD ₃ was more effective than VD ₂ to increase VD content of eggs in a way that after feeding the diet contain 6000 IU VD ₃ and D ₂ per kg feed, the VD content of egg yolk was 4.7 to 7.0 and 9.1 to 13 µg/100 g, respectively. Corresponding result for the diet containing 15000 IU/kg diet was 13.3 to 21 and 25.3 to 33.7 µg/100 g. The high VD fortified diet did not affect the production parameters such as Haugh unit, eggshell fracture, egg weight, and specific gravity. However, VD ₃ substantially promoted bone strength. The diets was not toxic for hens.	43
Chicken egg	Treatments: 43 µg VD ₃ /kg feed, 31 µg VD ₃ /kg feed + 30 µg 25(OH)D /kg feed, 55 µg 25(OH)D /kg feed, and 122 µg 25(OH)D /kg feed	60 weeks	All diets caused no significant change in egg-related parameters such as egg weight, specific gravity and eggshell fracture as well as hen-related parameters such as feed consumption ratio and live weight. In the diets containing only 25(OH)D, VD content were lower than 0.2 µg/100 g yolk.	44
Chicken egg	VD ₃ fortification: 9700, 17200, 24700, and 102200 IU/kg feed	40 weeks	The maximum VD ₃ levels in egg yolk were occurred at 3th weak and thereafter, their levels were stable. VD ₃ concentrations were 865, 1641, 2411, and 34815 IU/ 100 g yolk (wet basis) at 3th weak, respectively. There were no significant alterations in lipid profile, yolk viscosity and emulsification properties, sensory properties and hen performance after consumption of fortified diet.	45
Chicken egg	2500 to 10000 IU VD ₃ /kg feed	9 weeks	The diet did not significantly change hen performance and the physical properties of eggs.	14
Chicken egg	1) 1500 IU VD ₃ /kg feed 2) 3000 IU VD ₃ /kg feed 3) 1500 IU VD ₃ + 37.5 µg 25(OH)D/kg feed 4) 75 µg 25(OH)D/kg feed	8 weeks	The highest VD ₃ in egg yolk (5.06 µg/egg) was corresponded to diet containing 75 µg 25(OH)D/kg feed. The antioxidant activity of egg was improved in the 75 µg 25(OH)D/kg feed treatment. The diets did not significantly affected the egg production parameters.	46
Heifer	1) basal diet + 4000 IU VD ₃ /kg feed 2) basal diet + 4000 IU VD ₂ /kg feed 3) basal diet + 4000 IU VD ₂ -enriched mushrooms	30 days	Heifers that consumed VD ₃ containing diet had the highest content of VD ₃ in their meat as well as 25(OH)D in sera. The sensory properties, carcass attribute and meat quality did not alter as a result of consumption of the diets.	47
Pig's skin	-	-	LED-UV irradiation at 296 nm with the maximum applied dose of 20 kJ/m ² led to increment of VD ₃ content up to 3.5 to 4 µg/cm ² .	48
Rainbow trout (Oncorhynchus mykiss)	VD ₃ fortification: 1) 89 µg/kg feed 2) 174 µg/kg feed 3) 539 µg/kg feed	8 weeks	Elevating the dose of VD ₃ fortification did not improve the cholecalciferol content of muscle or liver of the fish.	49

bioavailability compared to that of VD₂. Feeding VD-deficient rats with bread fermented with UV-irradiated yeast as a rich source of VD₂ increased the serum 25(OH)D dose-dependently as the concentration of VD₂ increased in the bread.⁵⁰ However, the 25(OH)D serum concentration of rats fed with VD₃ supplemented bread was higher, indicating the higher bioavailability of VD₃ containing diets. Some studies indicate that comparing to VD₂, consuming VD₃ on a daily basis for a short period of time (like a few days) can initially rise serum 25(OH)D more and decrease 25(OH)D level more slightly over time in both men and women.^{51,52} while some other studies indicate almost similar concentration of 25(OH)D with insignificant differences after ingesting either VD₃ or VD₂ on a daily basis but for a longer time (like 11 weeks).^{53,54} Hence, continuously fortifying staple food products with either VD₃ or VD₂ does not necessarily or substantially affect VD bioavailability in populations.

Fortifying hens' feed with different concentrations of VD, reported promising results.⁴¹⁻⁴⁴ Additionally, the feed fortification with VD at levels mentioned in some studies was not toxic to hens. Also, the sensory and the lipid profile of eggs were unaffected due to VD fortification.

VD Stability During Household Processes

VD content declines in various foods during storage and other household processing as they affect VD stability in food products (see Table 3). Regular boiling and pan-frying processes led to 15-20% loss of VD₃ in eggs and baking wheat bread. However, processing foods in the oven at regular cooking temperature for 40 min leads to a great reduction of VD₃ for about 55-61%.¹⁶ Similar results were observed for VD₂. Thus, the total loss of vitamins varies and particularly depends on the chemical composition of the food matrix and processing situations such as time and temperature. Investigating the effects of different cooking methods like oven baking, pan-frying, steam cooking, and microwave cooking on the stability of VD₂ and VD₃ in rainbow trout fish, sunflower oil, and button mushrooms

indicated the highest VD loss in pan-frying, especially for button mushrooms.⁵⁵ Interestingly, VD retention increased when lemon juice was added, which could be due to its high antioxidant activity.⁵⁶ Since VD molecules are liable to oxygen,⁵⁷ the antioxidants in lemon juice prevented oxygen degradation. During long-term storage of milk powder (for 12 months), tremendous losses of VD₃ occurred owing to thermal isomerization⁵⁸ and anti-rachitic activity found only in *cis*-triene configuration VD as a bioactive compound while other isomers like *trans*-VD and tachysterol show little to no such activity.⁵⁹ VD deterioration possibly occurs when the food is processed with heat and kept in storage caused by the mechanism of VD isomerization to pre-VD form.⁶⁰ A way to compensate vitamin D loss might be to incorporate higher concentrations of VD in fortified food. However, as during food processing and storage, VD is lost in different amounts, potential risks of toxicity must be seriously considered. Hence, another way to address this problem could be to enhance the incremental stability of VD through encapsulation.

Encapsulation

Micro-nanoencapsulation refers to forming a barrier to prevent unfavorable chemical interactions and control the release of bioactive compounds. Based on the size of these small barriers, there are two main groups: microcapsules that are 5-300 µm in diameter,⁶¹ whereas nano-capsules are in the range of 50-1000 nm.^{18,62} The nano-scaled size contributes to higher solubility, better tissue permeability, prolonged clearance time, and improved cellular uptake of the entrapped compounds. Nano-sized capsules have remarkable benefits over the micro-sized ones, including excellent solubility, higher tissue permeability, protracted clearance time, and promoted cellular assimilation of the ensnared compounds.¹⁸ Thus, these advantages make nano-capsules as potential carriers for added food nutraceuticals. Based on recent investigations conducted to evaluate VD encapsulation (see Table 4), the efficiency depends on its size (micro or nano), utilized technique

Table 3. Influence of food processing on vitamin D.

Process Type	Food	Effect	Ref.
Pasteurization	Milk	No significant loss	63
Sterilization	Milk	No significant loss	63
Cooking	Beef	35-42% of the original vitamin D	64
Roasting	Beef	Significant loss	64
Frying	Mushroom, Egg and Margarine	Significant loss in ergocalciferol, 22-24% loss in vitamin D	16, 55
Boiling	Egg	Significant loss in 25- Hydroxycholecalciferol, 22-24% loss in vitamin D	16,65
Solar Drying	Fish meat, Fish oil	Significant loss	66
Steaming	Fish meat, Fish oil	Significant loss	67
Baking	Fish, meat, Bread	Significant reduction in Cholecalciferol, 24-31% loss in ergocalciferol	16,65,68
Oven Drying	Fish meal, Smoking Fish	Significant loss	69,70

(e.g., emulsification, precipitation, coacervation, electrospinning, and liposome), coating material (e.g., whey protein, casein, hydroxymethylcellulose) and core-to-wall ratio.¹⁸

VD Bioavailability

Effect of capsule size on VD bioavailability

Compared to micro-encapsulation, nano-encapsulation leads to higher bioavailability in nutraceuticals possibly due to the larger surface-to-volume proportion achieved in nano-encapsulation.⁷¹ Similarly, small-sized VD nano-

emulsions (233nm) had considerably higher cellular uptake and transport due to Caco-2 cell efficiencies compared to large-sized ones (350nm) and different surfactants, including soy lecithin, pea protein, and protein-lecithin, did not significantly affect the emulsion size.⁷² Under *in vitro* conditions, the bio-accessibility of VD₂ encapsulated in emulsions increased as the sizes of droplets decreased.⁷³ As the particle size declined, the surface-to-volume proportion increased, which resulted in accelerated interactions.⁷¹ In contrast, *in vivo* results demonstrated that the higher the droplet size, the better the bio-accessibility of

Table 4. Some studies conducted on micro/nanoencapsulation of VD.

Encapsulation type	Wall material	<i>In vitro</i> / <i>in vivo</i>	Encapsulation efficiency (%)	Main findings	Ref.
Microencapsulation	Medium molecular weight Manugel GHB Sodium alginate	<i>In vitro</i>	92	Improving the stability of VD ₂ during prolonged storage (5 months). Preserve the vitamin against gastric condition in a way that only 10% degradation were occurred. Complete proliferation of the vitamin under intestinal condition.	74
Microencapsulation	chitosan/ ethylcellulose complex	<i>In vitro</i>	86	Encapsulation could postpone VD ₂ liberation in gastric environment, but liberate the microencapsulated vitamin in intestine environment constantly over a prolonged time.	75
Microencapsulation	β-lactoglobulin and lysozyme	<i>In vitro</i> and <i>in vivo</i>	90.8 ± 4.8	After 4 weeks of storage at 4°C, the losses of VD ₃ were 91 and 44% for free VD ₃ and encapsulated ones, respectively. The stability of capsules to UV-C exposure was increase compared to free VD. Microencapsulation through β-lactoglobulin-lysozyme increased VD resistance in gastrointestinal stimulated condition as well as its bioavailability.	76
Microencapsulation	β-lactoglobulin	<i>In vitro</i>	-	The solubility of VD ₃ was significantly increased. The capsules exhibited high resistance in the pH ranged from 1.2 to 8.	77
Microencapsulation	β-lactoglobulin	<i>In vitro</i> and <i>in vivo</i>	94.5	The stability of BLG coagula containing VD ₃ under prolonged storage, exposure to UV-C and oxygen, and simulated intestinal fluid were higher than that of free VD. BLG coagula containing VD ₃ had increased stability against gastric proteases and might have different adsorption mechanism from free VD ₃ .	78
Microencapsulation	Whey protein isolate (WPI) and carboxymethyl cellulose (CMC)	<i>In vitro</i> and <i>in vivo</i>	92.1 to 93.8	The stability of VD ₃ in yoghurt and sour cream was significantly increased through by WPI and CMC emulsions during 14 days of storage at 4°C. The bioavailability of VD ₃ in emulsion forms was higher than that of its free form in mice samples.	79
Microencapsulation (composite gel)	Lotus root amylopectin and whey protein isolate	<i>In vitro</i> and <i>in vivo</i>	-	The composite gel increased the VD residence against gastrointestinal, UV light, long storage damages. The composite gels containing VD escalated serum 25(OH)D content more than 2 times compared to free VD.	80
Microencapsulation	Gelatin and cress seed mucilage	<i>In vitro</i> and <i>in vivo</i>	67.93	Ratios of mucilage to gelatin as well as core to shell influenced the load and efficiency of encapsulation. Encapsulation considerably increased the thermal stability of VD. 70% VD released simulated intestinal situation. Microencapsulation significantly improved the calcium and VD levels of blood in mice model.	81

Table 4. Continued.

Microencapsulation	5% WPI + 5% SPI	<i>In vitro</i>	> 95	About 50% VD release were occurred in stimulated intestinal fluid. The stability of VD were considerably influenced by wall material concentration and WPI to SPI ratio. VD retention during storage for 4 weeks was more than 93%	82
Nanocapsule	Carboxymethyl chitosan–soy protein complex	<i>In vitro</i>	96.8	Compared to soy protein isolate capsule: The release of VD in the gastric juice declined (42.3% compared to 86.1%). The release of VD in the intestinal condition increased (36.0% compared to 8.2%).	83
Nanoemulsions	Pea protein	<i>In vitro</i>	-	Corn oil (bioaccessibility = 73%) was suggested as the most suitable oil carrier. Monounsaturated-rich oils are better carriers than polyunsaturated-rich ones in terms of bioaccessibility and stability.	84
Nanoemulsions	Quillaja saponin emulsifier	<i>In vitro</i>	-	The bioaccessibility order based on different oil carriers: medium chain triglyceride (20%) < mineral oil (40%) < orange oil (70%) < corn oil (85%) ≈ fish oil (90%).	85
Nanoliposomes	Lecithin and cholesterol	-	> 93	The capsules were slightly liable to the presence of light and high temperature. Thus, it was suggested to preserve at dark and 4°C. No interaction was occurred between VD and wall materials.	86
Nanoliposomes	Oleic acid and glycerol monostearate	<i>In vitro</i>	85.6	Nanostructured lipid carriers (NLCs) of VD were stable during 20 days storage at 25°C. NLCs were stable in simulated gastric fluid. 90% proliferation of VD ₃ was observed in the stimulated intestinal condition.	87
Nanoprecipitation	Potato proteins	-	-	Nanocomplexation protected VD ₃ from thermal loss during pasteurization as well as from loss during simulated storage. The Nanocomplexed VD ₃ solution was transparent and could be used in various clear beverages.	88
Pickering emulsions	Nanofibrillated cellulose (NFC)	<i>In vitro</i>	-	The bioaccessibility of VD in simulated gastrointestinal fluids decreased as the content of NFC increased in the Pickering emulsion. Although the use of NFC in the emulsion might result in cluster formation, the droplets did not coagulate.	89
Nanocomplexation	Corn protein hydrolysate	<i>In vitro</i>	97	Under UV exposure, 30% of free VD retained, whereas the residue of encapsulated VD was up to 72%. The bioavailability of encapsulated VD was calculated to be up to 95%.	90
Nanocapsule	High amylose starch	<i>In vitro</i>	37.06–78.11	The vitamin release behavior in gastric condition was low, whereas it showed a quick initial release in intestinal simulant. Nanoencapsulation was able to improve the taste, homogeneity, and total acceptance of fortified milk.	91
Nanoemulsion	WPI	<i>In vitro</i>	-	The bioaccessibility of vitamin in simulated gastrointestinal condition was remarkably higher when the oil phase was digestible than when it was indigestible.	92
Nanoemulsion and nanocomplex	Soy protein isolate (SPI) + canola oil (for emulsion)	-	-	The Nanoemulsions and nanocomplexes of VD ₃ demonstrated a high stability against UV light-induced degradation.	93
Nanoemulsions	Fish oil	<i>In vitro</i>	95.7–98.2	The nanoemulsions had a shelf life of > 90 days. The nanoemulsions had antimicrobial activity against <i>Escherichia coli</i> and <i>Staphylococcus aureus</i> . The nanoemulsions displayed higher stability under gastrointestinal condition.	94

Table 4. Continued.

Nanocomplex	Ovalbumin and high methoxyl pectin	<i>In vitro</i>	96.37	Up to 80% VD release was occurred in intestinal fluid.	17
Nanoemulsion	Tween 80 and Soy lecithin	<i>In vitro</i>	-	Increasing storage temperature (4 vs 25 °C) decreased VD retention (74.4 vs 55.3) after 1 month.	95
Nanoemulsion	Pea protein isolate	<i>In vivo</i>	-	The VD deficient rats treated with nanoencapsulated VD had normal serum 25(OH) D level.	96
Nanoemulsion	Alginate and chitosan	<i>In vitro</i>	-	Co-encapsulation of VD ₃ and calcium decreased the bioavailability of VD.	97
Nanoemulsion	Pea protein	<i>In vitro</i>	94-96	Small-sized nanoemulsions (233 nm) had 2.5 and 5.3 times higher cellular uptake efficiency, and transport efficiency across Caco-2 cells than large-sized ones (350 nm), respectively.	47

VD₂. Higher bioavailability of large emulsions containing VD₂ might be ascribed to the increased pancreatic lipase secretion as large emulsions had higher contents of fats. Thus, a higher amount of lipid hydrolysis occurs, which leads to promoting VD₂ micellization and transport into the blood.

Effect of encapsulation technique on VD bioavailability

Encapsulation techniques may influence the bioavailability of VD. Assessing the resistance of VD₃, either in its free form or in β -lactoglobulin (BLG), under protracted storage, exposure to UV-C light, and oxygen conditions indicated that VD₃ in BLG complexes (proteins sensitive to pancreatic enzymes⁹⁸) was 3-5 times more stable than free VD₃.⁷⁸ Interestingly, the stability of BLG increased significantly after binding to VD, suggesting that this interaction had a dual and reciprocal preservative influence on both of the components. Under *in vivo* study, mice that were fed with BLG-VD complex demonstrated a significantly higher level of serum 25(OH)D than those fed with free VD₃. It was proposed that the BLG-VD complex had a different assimilation pathway from that of VD alone.⁵⁰ BLG-based coagulum containing VD₃ showed even greater resistance properties against gastric destructive conditions compared with the BLG-VD complex.⁹⁹

Effect of coating material on VD bioavailability

Utilizing disparate coating materials such as proteins, polysaccharides, and other types of surfactants in the encapsulation process resulted in different VD loading efficiencies and stabilities subsequently affecting the bioavailability of VD. The efficiency of BLG capsules encompassing VD₃ was assessed based on the effects of pH values in the range from 1.2 to 8 and concluded that different pH values did not affect the BLG-VD binding, which indicated the great potential of BLG to preserve VD in various food matrices and gastrointestinal tract conditions.⁷⁷ Besides, using BLG as a carrier for VD remarkably increased its solubility; furthermore, it was suggested that VD might bind to certain spots of BLG structure, including a region located at the pocket between

the α -helix and the β -barrel (as an exosite) and within the central calyx formed by the β -strands.^{100,101}

Evaluating the effectiveness of high amylose starch as a nanocarrier for VD₃ through simulated gastrointestinal tests showed that the proliferation of VD₃ was slight in the gastric situation, whereas a quick initial accumulation of VD occurred in the intestinal simulant. Moreover, sensory evaluation of milk containing either nanocarriers containing VD or VD in its free form suggested that the former obtained higher panel acceptability and improved homogeneity and taste compared to the latter. Free VD showed low solubility in milk. The high solubility of nanocarrier was ascribed to its small size.⁹¹ Although the high amylose starch nanocarrier containing VD showed great potential for application in milk. Its encapsulation efficiency was between 37.06 and 78.11, which was much lower than those of β -lactoglobulin microcapsules and carboxymethyl chitosan-soy protein nanocapsules.⁸³

Effect of core-to-wall ratio on VD bioavailability

oil-in-water nano-emulsion is created based on fish-oil containing VD₃ with a droplet size between 300 and 450 nm using ultrasonication technique. The encapsulation efficiency was increased from 95.7% to 98.2% as the oil concentration in emulsion increased.⁹⁴ The nano-emulsion solution was stable for more than 90 days. The simulated gastrointestinal tract assay showed higher resistance of nano-emulsions with increased oil concentration than those of low-oil-concentration against dangerous conditions. Creating a thicker oil layer around VD in emulsions containing a higher oil ratio resulted in reinforced mechanical strength against the acidic condition of the stomach. Both core-to-cell ratio and cress seed gum to gelatin ratio remarkably affect the encapsulation and loading efficiencies of encapsulated VD through complex coacervation.⁸¹ The simulated gastrointestinal test revealed that 70% VD proliferated from capsules in the intestine. Moreover, *in vivo* results suggested that microencapsulated VD effectively increased VD blood content.

Effect of carrier type on VD bioavailability

Investigating the impact of the type of carrier oil, including fish, flaxseed, or corn oil on the bioavailability and stability of VD₃ nano-emulsions *in vitro* gastrointestinal tract test revealed that the oil type significantly affected the bioavailability of VD₃. Corn oil (bioavailability=73%) was suggested as the most suitable carrier oil than the others (bioavailability ~ 43%), indicating the higher desirability of monounsaturated-rich oils than polyunsaturated-rich ones as far as stability and bioaccessibility.⁸⁷ Furthermore, long-chain triglycerides could be more appropriate carriers than medium-chain ones for VD₃ delivery.⁸⁸ This could be ascribed to the higher capacity of mixed micelles created by free fatty acids to solubilize VD.

Evaluating the bio-accessibility of mineral oil (indigestible oil) as a carrier for VD₃ comparing to corn oil (digestible oil) suggested that the bio-accessibility of VD₃ remarkably declined when mineral oil was used as a carrier under simulated gastrointestinal conditions.⁹² VD₃ was inclined to maintain ensnared inside the droplets of mineral oil even throughout the protracted storage under gastrointestinal conditions. The bio-accessibility of VD₃ carried by corn oil reached culmination after half an hour, and then the bio-accessibility gradually decreased. It is possible that during prolonged storage in the simulated intestinal fluid, some interactions may occur between anionic micelles containing VD₃ and cationic calcium ions leading to an increase in both particle size and sedimentation. From these observations, it is deduced that carrier oil composition, micelle aggregation, and micelle solubilization can significantly influence VD₃'s bioavailability.

Safety Consideration

The risk of overdose in VD consumption has always been a concern. In one extreme, consuming VD-containing foods or supplements are recommended as they increase the 25(OH)D up to 50 to 75 nmol/L. At the other extreme, the upper level for serum 25(OH)D was considered as 225 nmol/L.¹⁰² However, consuming more VD would result in some detrimental side effects. Besides, the upper intake level for VD₃ consumption is about 4000 IU/day (= 100 µg VD₃).¹⁰³ However, the last level is far beyond what is fortified in foods in order to reach the optimum level of serum 25(OH)D.

Another concern is about using nanoparticles in food products and their possible harms in case of ingestion. Most nanomaterials enter the intestine via oral ingestion, and assimilation through enterocytes is blueprinted not to permit foreign or large particles to transport across them; nonetheless, the nano-sized components can pass through these barriers; thus there is a potential risk to cause gastrointestinal disorders.¹⁸ Moreover, the destiny of undigested nano-capsules, which are assimilated into the bloodstream, is still unknown. Consuming foods containing nanomaterials should be considered health-threatening unless the investigations evince otherwise.¹⁰⁴

Thus, more clinical and *in vivo* studies should be carried out about different health-related aspects of using nanoencapsulation in the food industry.

Conclusion

VD₃ deficiency is highly prevalent among the world populations, and the new dietary VD sources make it unfeasible for most children and adults to meet the recommended level of intake. Based on reviewed investigations, sustainable food-based guidelines can be offered to bridge the gap between new and suggested intakes of VD while preventing the risk of habitual extortionate intakes.

Fortification or supplementation of food products with VD has a low risk of toxicity. Since the uptake was typically low, VD supplementation would not be efficient at a population level, hence, innovative food-based solutions were required to bridge the gap between the new requirement values and the new intakes. For this purpose, direct fortification and biofortification are two promising approaches to meet the intended goals. For adequate intake, some strategies are proposed, such as: improving food labeling about natural and added VD concentrations and compelling the manufacturers to fortify various staple foods by shifting the regulations from optional to compulsory fortification.

The risk of overdose because of fortifying a single staple product is higher than the risk associated with fortifying various food products. Thus, we suggest that several staple food products should be fortified with VD to meet the requirements for the population. As VD loss of VD in household food processing is remarkable and relatively unavoidable, encapsulation techniques will be advantageous to increase VD's resistance against various detrimental situations during processing. Thus, this technique offers great potential to expand the spectrum of food products that can be fortified due to their unique specifications. Furthermore, different encapsulation techniques showed promising outcomes as far as VD bioavailability. However, more investigations must be conducted on optimizing the procedures to encapsulate VD to be more applicable for a particular product. Although the nanoencapsulation of VD displayed satisfactory results, the safety of using these materials for human ingestion is still obscure. Thus, to illustrate the exact effects of nanoencapsulation on the body, more comprehensive studies under *in vivo* conditions must be done.

Author Contributions

ME and RY: drafting the work, design of the work; the acquisition, analysis. AA, KG, RZD, and AH: conception, and interpretation of data for the work. ZSD, MA, and MM: revising the manuscript. All the authors agreed to the published version of the manuscript.

Conflict of Interest

The authors report no conflicts of interest.

References

1. Spiro A, Buttriss JL. Vitamin D: An overview of vitamin D status and intake in Europe. Vol. 39, Nutrition Bulletin. 2014;39(4):322-50. doi:10.1111/nbu.12108
2. Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? J Steroid Biochem Mol Biol. 2014;144(PART A):138-45. doi:10.1016/j.jsbmb.2013.11.003
3. Maurya VK, Aggarwal M. Factors influencing the absorption of vitamin D in GIT: an overview. J Food Sci Technol. 2017;54(12):3753-65. doi:10.1007/s13197-017-2840-0.
4. Lips P. Worldwide status of vitamin D nutrition. J Steroid Biochem Mol Biol. 2010;121(1-2):297-300. doi:10.1016/j.jsbmb.2010.02.021
5. Vieth R. The Pharmacology of Vitamin D, Including Fortification Strategies. Vitamin D. Cambridge, Massachusetts: Academic Press; 2005. p. 995-1015. doi:10.1016/B978-012252687-9/50064-4
6. Feldman D, Krishnan A V, Swami S, Giovannucci E, Feldman BJ. The role of vitamin D in reducing cancer risk and progression. Nat Rev Cancer. 2014;14(5):342-57. doi:10.1038/nrc3691
7. Jamshidinaeini Y, Akbari ME, Abdollahi M, Ajami M, Davoodi SH. Vitamin D status and risk of breast cancer in Iranian women: A case-control study. J Am Coll Nutr. 2016;35(7):639-46. doi:10.1080/07315724.2015.1127786
8. Rimmelzwaan LM, Van Schoor NM, Lips P, Berendse HW, Eekhoff EMW. Systematic Review of the Relationship between Vitamin D and Parkinson's Disease. J Parkinsons Dis. 2016;6(1):29-37. doi:10.3233/JPD-150615
9. Kočovská E, Gaughran F, Krivoy A, Meier UC. Vitamin-D deficiency as a potential environmental risk factor in multiple sclerosis, schizophrenia, and autism. Front Psychiatry. 2017;8:47. doi:10.3389/fpsy.2017.00047
10. Al Mheid I, Quyyumi AA. Vitamin D and Cardiovascular Disease: Controversy Unresolved. J Am Coll Cardiol. 2017;70(1):89-100. doi:10.1016/j.jacc.2017.05.031
11. Vosoughi A, Aliasgarzadeh A, Bahrami A, Abbasalizadeh F, Niafar M, Najafipour F, et al. Concentration of maternal serum 25-hydroxy Vitamin D and gestational 2 diabetes mellitus risk. Pharm Sci. 2017;23(3):189-92. doi:10.15171/PS.2017.28
12. Chirumbolo S, Bjørklund G, Sboarina A, Vella A. The role of vitamin D in the immune system as a pro-survival molecule. Clin Ther. 2017;39(5):894-916. doi:10.1016/j.clinthera.2017.03.021
13. Adolphe JL, Whiting SJ, Dahl WJ. Vitamin fortification of pureed foods for long-term care residents. Can J Diet Pract Res. 2009;70(3):143-50. doi:10.3148/70.3.2009.143
14. Browning LC, Cowieson AJ. Vitamin D fortification of eggs for human health. J Sci Food Agric. 2014;94(7):1389-96. doi:10.1002/jsfa.6425
15. Guo J, Lovegrove JA, Givens DI. Food fortification and biofortification as potential strategies for prevention of vitamin D deficiency. Nutr Bull. 2019;44(1):36-42. doi:10.1111/nbu.12363
16. Jakobsen J, Knuthsen P. Stability of vitamin D in foodstuffs during cooking. Food Chem. 2014;148:170-5. doi:10.1016/j.foodchem.2013.10.043
17. Xiang C, Gao J, Ye H, Ren G, Ma X, Xie H, et al. Development of ovalbumin-pectin nanocomplexes for vitamin D3 encapsulation: Enhanced storage stability and sustained release in simulated gastrointestinal digestion. Food Hydrocoll. 2020;106:105926. doi:10.1016/j.foodhyd.2020.105926
18. Katouzian I, Jafari SM. Nano-encapsulation as a promising approach for targeted delivery and controlled release of vitamins. Trends Food Sci Technol. 2016;53:34-48. doi:10.1016/j.tifs.2016.05.002
19. Verkaik-Kloosterman J, Seves SM, Ocké MC. Vitamin D concentrations in fortified foods and dietary supplements intended for infants: Implications for vitamin D intake. Food Chem. 2017;221:629-35. doi:10.1016/j.foodchem.2016.11.128
20. Norouzebeigi S, Vahid-Dastjerdi L, Yekta R, Sohrabvandi S, Zendeboodi F, Mortazavian AM. Celiac therapy by administration of probiotics in food products: a review. Curr Opin Food Sci. 2020;32:58-66. doi:10.1016/j.cofs.2020.01.005
21. Wood RA, Kim JS, Lindblad R, Nadeau K, Henning AK, Dawson P, et al. A randomized, double-blind, placebo-controlled study of omalizumab combined with oral immunotherapy for the treatment of cow's milk allergy. J Allergy Clin Immunol. 2016;137(4):1103-10. doi:10.1016/j.jaci.2015.10.005
22. Tangpricha V, Koutkia P, Rieke SM, Chen TC, Perez AA, Holick MF. Fortification of orange juice with vitamin D: A novel approach for enhancing vitamin D nutritional health. Am J Clin Nutr. 2003;77(6):1478-83. doi:10.1093/ajcn/77.6.1478
23. Johnson JL, Mistry VV, Vukovich MD, Hogie-Lorenzen T, Hollis BW, Specker BL. Bioavailability of vitamin D from fortified process cheese and effects on vitamin D status in the elderly. J Dairy Sci. 2005;88(7):2295-301. doi:10.3168/jds.S0022-0302(05)72907-6
24. Semo E, Kesselman E, Danino D, Livney YD. Casein micelle as a natural nano-capsular vehicle for nutraceuticals. Food Hydrocoll. 2007;21(5-6):936-42. doi:10.1016/j.foodhyd.2006.09.006
25. Onal H, Adal E, Alpaslan S, Ersen A, Aydin A. Is daily 400 IU of vitamin D supplementation appropriate for every country: A cross-sectional study. Eur J Nutr. 2010;49(7):395-400. doi:10.1007/s00394-010-0097-8
26. Khadgawat R, Marwaha RK, Garg MK, Ramot R, Oberoi AK, Sreenivas V, et al. Impact of vitamin D fortified milk supplementation on vitamin D status of

- healthy school children aged 10-14 years. *Osteoporos Int.* 2013;24(8):2335-43. doi:10.1007/s00198-013-2306-9
27. Allen RE, Dangour AD, Tedstone AE, Chalabi Z. Does fortification of staple foods improve vitamin D intakes and status of groups at risk of deficiency? A United Kingdom modeling study. *Am J Clin Nutr.* 2015;102(2):338-44. doi:10.3945/ajcn.115.107409
 28. Jääskeläinen T, Itonen ST, Lundqvist A, Erkkola M, Koskela T, Lakkala K, et al. The positive impact of general Vitamin D food fortification policy on Vitamin D status in a representative adult Finnish population: Evidence from an 11-y follow-up based on standardized 25-hydroxyVitamin D data. *Am J Clin Nutr.* 2017;105(6):1512-20. doi:10.3945/ajcn.116.151415
 29. Cashman KD, O'Dea R. Exploration of strategic food vehicles for vitamin D fortification in low/lower-middle income countries. *J Steroid Biochem Mol Biol.* 2019;195:105479. doi:10.1016/j.jsbmb.2019.105479
 30. Wilson LR, Tripkovic L, Hart KH, Lanham-New SA. Vitamin D deficiency as a public health issue: Using Vitamin D2 or Vitamin D3 in future fortification strategies. *Proc Nutr Soc.* 2017;76(3):392-9. doi:10.1017/S0029665117000349
 31. Banville C, Vuilleumard JC, Lacroix C. Comparison of different methods for fortifying Cheddar cheese with vitamin D. *Int Dairy J.* 2000;10(5-6):375-82. doi:10.1016/S0958-6946(00)00054-6
 32. Wagner D, Sidhom G, Whiting SJ, Rousseau D, Vieth R. The bioavailability of vitamin d from fortified cheeses and supplements is equivalent in adults. *J Nutr.* 2008;138(7):1365-71. doi:10.1093/jn/138.7.1365
 33. Wagner D, Rousseau D, Sidhom G, Pouliot M, Audet P, Vieth R. Vitamin D3 fortification, quantification, and long-term stability in cheddar and low-fat cheeses. *J Agric Food Chem.* 2008;56(17):7964-9. doi:10.1021/jf801316q
 34. Ganesan B, Brotherson C, McMahon DJ. Fortification of Cheddar cheese with vitamin D does not alter cheese flavor perception. *J Dairy Sci.* 2011;94(7):3708-14. doi:10.3168/jds.2010-4020
 35. Kazmi SA, Vieth R, Rousseau D. Vitamin D3 fortification and quantification in processed dairy products. *Int Dairy J.* 2007;17(7):753-9. doi:10.1016/j.idairyj.2006.09.009
 36. Hanson AL, Metzger LE. Evaluation of increased vitamin D fortification in high-temperature, short-time-processed 2% milk, UHT-processed 2% fat chocolate milk, and low-fat strawberry yogurt. *J Dairy Sci.* 2010;93(2):801-7. doi:10.3168/jds.2009-2694
 37. Mc Kenna MJ, Freaney R, Byrne P, Mc Brinn Y, Murray B, Kelly M, et al. Safety and efficacy of increasing wintertime vitamin d and calcium intake by milk fortification. *Qjm.* 1995;88(12):895-8.
 38. Biancuzzo RM, Young A, Bibuld D, Cai MH, Winter MR, Klein EK, et al. Fortification of orange juice with vitamin D2 or vitamin D3 is as effective as an oral supplement in maintaining vitamin D status in adults. *Am J Clin Nutr.* 2010;91(6):1621-6. doi:10.3945/ajcn.2009.27972
 39. Upreti P, Mistry VV, Warthesen JJ. Estimation and fortification of vitamin D3 in pasteurized process cheese. *J Dairy Sci.* 2002;85(12):3173-81. doi:10.3168/jds.S0022-0302(02)74405-6
 40. Jakobsen J, Maribo H, Bysted A, Sommer HM, Hels O. 25-Hydroxyvitamin D3 affects vitamin D status similar to vitamin D3 in pigs - But the meat produced has a lower content of vitamin D. *Br J Nutr.* 2007;98(5):908-13. doi:10.1017/S0007114507756933
 41. Mattila P, Lehtikainen K, Kiiskinen T, Piironen V. Cholecalciferol and 25-hydroxycholecalciferol content of chicken egg yolk as affected by the cholecalciferol content of feed. *J Agric Food Chem.* 1999;47(10):4089-92. doi:10.1021/jf990183c
 42. Mattila P, Rokka T, Könkö K, Valaja J, Rossow L, Ryhänen EL. Effect of cholecalciferol-enriched hen feed on egg quality. *J Agric Food Chem.* 2003;51(1):283-7. doi:10.1021/jf020743z
 43. Mattila P, Valaja J, Rossow L, Venäläinen E, Tupasela T. Effect of vitamin D2- and D3-enriched diets on egg vitamin D content, production, and bird condition during an entire production period. *Poult Sci.* 2004;83(3):433-40. doi:10.1093/ps/83.3.433
 44. Mattila PH, Valkonen E, Valaja J. Effect of different vitamin d supplementations in poultry feed on vitamin d content of eggs and chicken meat. *J Agric Food Chem.* 2011;59(15):8298-303. doi:10.1021/jf2012634
 45. Yao L, Wang T, Persia M, Horst RL, Higgins M. Effects of Vitamin D3-Enriched Diet on Egg Yolk Vitamin D3 Content and Yolk Quality. *J Food Sci.* 2013;78(2):178-83. doi:10.1111/1750-3841.12032
 46. Duffy SK, Rajauria G, Clarke LC, Kelly AK, Cashman KD, O'Doherty JV. The potential of cholecalciferol and 25-hydroxyvitamin D3 enriched diets in laying hens, to improve egg vitamin D content and antioxidant availability. *Innov Food Sci Emerg Technol.* 2017;44:109-16. doi: 10.1016/j.ifset.2017.07.007
 47. Duffy SK, O'Doherty J V., Rajauria G, Clarke LC, Hayes A, Dowling KG, et al. Vitamin D-biofortified beef: A comparison of cholecalciferol with synthetic versus UVB-mushroom-derived ergosterol as feed source. *Food Chem.* 2018;256:18-24. doi:10.1016/j.foodchem.2018.02.099
 48. Barnkob LL, Argyraki A, Petersen PM, Jakobsen J. Investigation of the effect of UV-LED exposure conditions on the production of Vitamin D in pig skin. *Food Chem.* 2016;212:386-91. doi:10.1016/j.foodchem.2016.05.155
 49. Mattila P, Piironen V, Hakkarainen T, Hirvi T, Uusi-Rauva E, Eskelinen P. Possibilities to raise vitamin D content of rainbow trout (*Oncorhynchus mykiss*) by elevated feed cholecalciferol contents. *J Sci Food Agric.* 1999;79(2):195-8. doi:10.1002/(sici)1097-

- 0010(199902)79:2<195::aid-jsfa166>3.0.co;2-c
50. Hohman EE, Martin BR, Lachcik PJ, Gordon DT, Fleet JC, Weaver CM. Bioavailability and efficacy of vitamin D2 from UV-irradiated yeast in growing, vitamin D-deficient rats. *J Agric Food Chem.* 2011;59(6):2341-6. doi:10.1021/jf104679c
 51. Romagnoli E, Mascia ML, Cipriani C, Fassino V, Mazzei F, D'Erasmus E, et al. Short and long-term variations in serum calciotropic hormones after a single very large dose of ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) in the elderly. *J Clin Endocrinol Metab.* 2008;93(8):3015-20. doi:10.1210/jc.2008-0350
 52. Armas LAG, Hollis BW, Heaney RP. Vitamin D2 is much less effective than vitamin D3 in humans. *J Clin Endocrinol Metab.* 2004;89(11):5387-91. doi:10.1210/jc.2004-0360
 53. Holick MF, Biancuzzo RM, Chen TC, Klein EK, Young A, Bibuld D, et al. Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. *J Clin Endocrinol Metab.* 2008;93(3):677-81. doi:10.1210/jc.2007-2308
 54. Biancuzzo RM, Young A, Bibuld D, Cai MH, Winter MR, Klein EK, et al. Fortification of orange juice with vitamin D2 or vitamin D3 is as effective as an oral supplement in maintaining vitamin D status in adults. *Am J Clin Nutr.* 2010;91(6):1621-6. doi:10.3945/ajcn.2009.27972
 55. Ložnjak P, Jakobsen J. Stability of vitamin D3 and vitamin D2 in oil, fish and mushrooms after household cooking. *Food Chem.* 2018;254:144-9. doi: 10.1016/j.foodchem.2018.01.182
 56. Hajimahmoodi M, Aliabadipoor M, Moghaddam G, Sadeghi N, Oveisi MR, Jannat B. Evaluation of in vitro antioxidant activities of lemon juice for safety assessment. *Am J Food Technol.* 2012;7:708-14. doi:10.3923/ajft.2012.708.714
 57. Hemery YM, Fontan L, Moench-Pfanner R, Laillou A, Berger J, Renaud C, et al. Influence of light exposure and oxidative status on the stability of vitamins A and D3 during the storage of fortified soybean oil. *Food Chem.* 2015;184:90-8. doi:10.1016/j.foodchem.2015.03.096
 58. Mahmoodani F, Perera CO, Abernethy G, Fedrizzi B, Chen H. Lipid oxidation and vitamin D3 degradation in simulated whole milk powder as influenced by processing and storage. *Food Chem.* 2018;261:149-56. doi:10.1016/j.foodchem.2018.04.043
 59. Mahmoodani F, Perera CO, Fedrizzi B, Abernethy G, Chen H. Degradation studies of cholecalciferol (vitamin D3) using HPLC-DAD, UHPLC-MS/MS and chemical derivatization. *Food Chem.* 2017;219:373-81. doi:10.1016/j.foodchem.2016.09.146
 60. Donald BP. Determination of vitamin k in foods: A review. *Crit Rev Food Sci Nutr.* 1980;13(4):337-52. doi:10.1080/10408398009527294
 61. Gibbs BF, Kermasha S, Alli I, Mulligan CN. Encapsulation in the food industry: A review. *Int J Food Sci Nutr.* 1999;50(3):213-24. doi:10.1080/096374899101256
 62. Yekta R, Mirmoghtadaie L, Hosseini H, Norouzbeigi S, Hosseini SM, Shojaee-Aliabadi S. Development and characterization of a novel edible film based on *Althaea rosea* flower gum: Investigating the reinforcing effects of bacterial nanocrystalline cellulose. *Int J Biol Macromol.* 2020;158:327-37. doi:10.1016/j.ijbiomac.2020.04.021
 63. Richardson T, Finley JW. Chemical changes in food during processing. Berlin: Springer Science & Business Media; 2012. doi:10.1007/978-1-4613-2265-8
 64. Bennink MR, Ono K. Vitamin B12, E and D content of raw and cooked beef. *J Food Sci.* 1982;47(6):1786-92. doi:10.1111/j.1365-2621.1982.tb12883.x
 65. Mattila P, Ronkainen R, Lehtikainen K, Piironen V. Effect of household cooking on the vitamin d content in fish, eggs, and wild mushrooms. *J Food Compos Anal.* 1999;12(3):153-60. doi:10.1006/jfca.1999.0828
 66. Suzuki H, Hayakawa S, Wada S, Okazaki E, Yamazawa M. Effect of solar drying on vitamin D3 and provitamin D3 contents in fish meat. *J Agric Food Chem.* 1988;36(4):803-6. doi: 10.1021/jf00082a033
 67. Scott KC, Latshaw JD. Effects of commercial processing on the fat-soluble vitamin content of menhaden fish oil. *J Am Oil Chem Soc.* 1991;68(4):234-6. doi:10.1007/BF02657615
 68. Mawer B, Gomes U. Estimation of vitamin D and its metabolites in meat. In: Norman AW, Bouillon R, Thomasset M. editors, *Vitamin D, Proceedings of the Ninth Workshop on Vitamin D, Orlando, Florida, 28 May-2 June 1994.* Berlin: Walter de Gruyter, p.775-6.
 69. Scott KC, Latshaw JD. The vitamin D3 and precholecalciferol content of menhaden fish meal as affected by drying conditions. *Anim Feed Sci Technol.* 1994;47(1-2):99-105. doi:10.1016/0377-8401(94)90163-5
 70. Aminullah Bhuiyan AKM, Ratnayake WMN, Ackman RG. Nutritional composition of raw and smoked atlantic mackerel (*scomber scombrus*): Oil- and water-soluble vitamins. *J. Food Compos. Anal.* 1993;6(2):172-84. doi:10.1006/jfca.1993.1019
 71. Li Y, McClements DJ. New mathematical model for interpreting pH-stat digestion profiles: Impact of lipid droplet characteristics on in vitro digestibility. *J Agric Food Chem.* 2010;58(13):8085-92. doi:10.1021/jf101325m
 72. Walia N, Chen L. Pea protein based vitamin D nanoemulsions: Fabrication, stability and in vitro study using Caco-2 cells. *Food Chem.* 2020;305:125475. doi:10.1016/j.foodchem.2019.125475
 73. Salvia-Trujillo L, Fumiaki B, Park Y, McClements DJ. The influence of lipid droplet size on the oral bioavailability of Vitamin D2 encapsulated in emulsions: an in vitro and in vivo study. *Food Funct.* 2017;8(2):767-77. doi:10.1039/c6fo01565d

74. Barba AA, Dalmoro A, d'Amore M, Lamberti G. Liposoluble vitamin encapsulation in shell-core microparticles produced by ultrasonic atomization and microwave stabilization. *LWT - Food Sci Technol.* 2015;64(1):149-56. doi:10.1016/j.lwt.2015.05.040
75. Shi X, Tan T. Preparation of complex chitosan microcapsule and its application in controlled release of vitamin D2. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi.* 2003;20(1):26-9.
76. Diarrassouba F, Garrait G, Remondetto G, Alvarez P, Beyssac E, Subirade M. Food protein-based microspheres for increased uptake of Vitamin D3. *Food Chem.* 2015;173:1066-72. doi:10.1016/j.foodchem.2014.10.112
77. Diarrassouba F, Remondetto G, Liang L, Garrait G, Beyssac E, Subirade M. Effects of gastrointestinal pH conditions on the stability of the β -lactoglobulin/vitamin D3 complex and on the solubility of vitamin D3. *Food Res Int.* 2013;52(2):515-21. doi:10.1016/j.foodres.2013.02.026
78. Diarrassouba F, Garrait G, Remondetto G, Alvarez P, Beyssac E, Subirade M. Increased stability and protease resistance of the β -lactoglobulin/vitamin D3 complex. *Food Chem.* 2014;145:646-52. doi:10.1016/j.foodchem.2013.08.075
79. Leskauskaite D, Jasutiene I, Malinauskite E, Kersiene M, Matusevicius P. Fortification of dairy products with vitamin D3. *Int J Dairy Technol.* 2016;69(2):177-83. doi:10.1111/1471-0307.12242
80. Liu K, Kong XL, Li QM, Zhang HL, Zha XQ, Luo JP. Stability and bioavailability of vitamin D3 encapsulated in composite gels of whey protein isolate and lotus root amylopectin. *Carbohydr Polym.* 2020;227:115337. doi:10.1016/j.carbpol.2019.115337
81. Jannasari N, Fathi M, Moshtaghian SJ, Abbaspourrad A. Microencapsulation of vitamin D using gelatin and cress seed mucilage: Production, characterization and in vivo study. *Int J Biol Macromol.* 2019;129:972-9. doi:10.1016/j.ijbiomac.2019.02.096
82. Khan WA, Butt MS, Pasha I, Jamil A. Microencapsulation of vitamin D in protein matrices: in vitro release and storage stability. *J Food Meas Charact.* 2020;14(3):1172-82. doi:10.1007/s11694-019-00366-3
83. Teng Z, Luo Y, Wang Q. Carboxymethyl chitosan-soy protein complex nanoparticles for the encapsulation and controlled release of vitamin D3. *Food Chem.* 2013;141(1):524-32. doi:10.1016/j.foodchem.2013.03.043
84. Schoener AL, Zhang R, Lv S, Weiss J, McClements DJ. Fabrication of plant-based vitamin D3 -fortified nanoemulsions: Influence of carrier oil type on vitamin bioaccessibility. *Food Funct.* 2019;10(4):1826-35. doi:10.1039/C9FO00116F
85. Mohammadi M, Ghanbarzadeh B, Hamishehkar H. Formulation of nanoliposomal vitamin D3 for potential application in beverage fortification. *Adv Pharm Bull.* 2014;4(Suppl 2):569-75. doi:10.5681/apb.2014.084
86. Ozturk B, Argin S, Ozilgen M, McClements DJ. Nanoemulsion delivery systems for oil-soluble vitamins: Influence of carrier oil type on lipid digestion and vitamin D3 bioaccessibility. *Food Chem.* 2015;187:499-506. doi:10.1016/j.foodchem.2015.04.065
87. Park SJ, Garcia CV, Shin GH, Kim JT. Development of nanostructured lipid carriers for the encapsulation and controlled release of vitamin D3. *Food Chem.* 2017;225:213-9. doi:10.1016/j.foodchem.2017.01.015
88. David S, Livney YD. Potato protein based nanovehicles for health promoting hydrophobic bioactives in clear beverages. *Food Hydrocoll.* 2016;57:229-35. doi:10.1016/j.foodhyd.2016.01.027
89. Winuprasith T, Khomein P, Mitbumrung W, Suphantharika M, Nitithamyong A, McClements DJ. Encapsulation of vitamin D3 in pickering emulsions stabilized by nanofibrillated mangosteen cellulose: Impact on in vitro digestion and bioaccessibility. *Food Hydrocoll.* 2018;83:153-64. doi:10.1016/j.foodhyd.2018.04.047
90. Lin Y, Wang YH, Yang XQ, Guo J, Wang JM. Corn protein hydrolysate as a novel nano-vehicle: Enhanced physicochemical stability and in vitro bioaccessibility of vitamin D3. *LWT - Food Sci Technol.* 2016;72:510-7. doi:10.1016/j.lwt.2016.05.020
91. Hasanvand E, Fathi M, Bassiri A, Javanmard M, Abbaszadeh R. Novel starch based nanocarrier for Vitamin D fortification of milk: Production and characterization. *Food Bioprod. Process.* 2015;96:264-77. doi:10.1016/j.fbp.2015.09.007
92. Tan Y, Liu J, Zhou H, Muriel Mundo J, McClements DJ. Impact of an indigestible oil phase (mineral oil) on the bioaccessibility of vitamin D3 encapsulated in whey protein-stabilized nanoemulsions. *Food Res Int.* 2019;120:264-74. doi:10.1016/j.foodres.2019.02.031
93. Lee H, Yildiz G, dos Santos LC, Jiang S, Andrade JE, Engeseth NJ, et al. Soy protein nano-aggregates with improved functional properties prepared by sequential pH treatment and ultrasonication. *Food Hydrocoll.* 2016;55:200-9. doi:10.1016/j.foodhyd.2015.11.022
94. Walia N, Dasgupta N, Ranjan S, Chen L, Ramalingam C. Fish oil based vitamin D nanoencapsulation by ultrasonication and bioaccessibility analysis in simulated gastro-intestinal tract. *Ultrason Sonochem.* 2017;39:623-35. doi:10.1016/j.ultsonch.2017.05.021
95. Mehmood T, Ahmed A. Tween 80 and Soya-Lecithin-Based Food-Grade Nanoemulsions for the Effective Delivery of Vitamin D. *Langmuir.* 2020;36(11):2886-92. doi:10.1021/acs.langmuir.9b03944
96. Almajwal AM, Abulmeaty MMA, Feng H, Alruwaili NW, Dominguez-Uscanga A, Andrade JE, et al. Stabilization of vitamin D in pea protein isolate nanoemulsions increases its bioefficacy in rats. *Nutrients.* 2019;11(1):75. doi:10.3390/nu11010075

97. Dima C, Dima S. Bioaccessibility study of calcium and vitamin D₃ co-microencapsulated in water-in-oil-in-water double emulsions. *Food Chem.* 2020;303(15):125369. doi:10.1016/j.foodchem.2019.125416
98. Creamer LK, Nilsson HC, Paulsson MA, Coker CJ, Hill JP, Jiménez-Flores R. Effect of genetic variation on the tryptic hydrolysis of bovine β -lactoglobulin A, B, and C. *J Dairy Sci.* 2004;87(12):4023-32. doi:10.3168/jds.S0022-0302(04)73543-2
99. Diarrassouba F, Garrat G, Remondetto G, Alvarez P, Beyssac E, Subirade M. Improved bioavailability of vitamin D₃ using a β -lactoglobulin-based coagulum. *Food Chem.* 2015;172:361-7. doi:10.1016/j.foodchem.2014.09.054
100. Yang MC, Guan HH, Yang JM, Ko CN, Liu MY, Lin YH, et al. Rational design for crystallization of β -lactoglobulin and vitamin D₃ complex: Revealing a secondary binding site. *Cryst Growth Des.* 2008;8(12):4268-76. doi:10.1021/cg800697s
101. Yang MC, Guan HH, Liu MY, Lin YH, Yang JM, Chen WL, et al. Crystal structure of a secondary vitamin D₃ binding site of milk β -lactoglobulin. *Proteins.* 2008;71(3):1197-210. doi:10.1002/prot.2181
102. Vieth R. Implications for 25-hydroxyvitamin D testing of public health policies about the benefits and risks of Vitamin D fortification and supplementation. *Scand J Clin Lab Invest Suppl.* 2012;243:144-53. doi:10.3109/00365513.2012.682893
103. Ritu G, Gupta A, Gupta A. Fortification of foods with vitamin D in India. *Nutrients.* 2014;6(9):3601-23. doi:10.3390/nu6093601
104. Babazadeh A, Jafari SM, Shi B. Encapsulation of food ingredients by nanophytosomes. In: Jafari SM, editor, *Lipid-Based Nanostructures for Food Encapsulation Purposes.* Amsterdam: Elsevier; 2019. p. 405-43. doi: 10.1016/B978-0-12-815673-5.00010-6