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Encapsulating vitamin D: a feasible and promising approach to combat its deficiency

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Running title: Effective factors on vitamin D encapsulation

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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.

Keywords: Encapsulation, Vitamin D, Fortification, Bioavailability, Enrichment, Food.

Introduction

Vitamin D (VD) is a lipid-soluble vitamin comprised of ergocalciferol (VD_2) and cholecalciferol (VD_3). VD_2 is naturally synthesized in plant resources such as fungi and yeasts, whereas VD_3 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 VD_3 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 VD_2/240 ml whole milk or 25000 IU VD_2/240 ml skim milk and toast containing 25000 IU VD_2[22] showed that milk's fat content did not influence the bioavailability of VD in peak serum VD_2 contents. However, when VD_2 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 VD_3 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 VD_3 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 VD_3/100g 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, VD_3 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 VD_3 had a higher bioavailability than VD_2 thus, fortifying foods with VD_3 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 VD_3 could be considered an effective approach to fighting against VD deficiency in the populations.

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 VD_3, mixtures of VD_3 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 VD_3 increased in the diet.[31] Furthermore, if 25(OH)D was added as complementary (at lower ratio) to VD_3 in the pigs' diet, the $25(OH)D_3$ concentration of serum, liver, and meat could be equally affected by the concentration of both components.[31]

Lots of discrepancies are among studies on VD_3 bioavailability compared to that of VD_2. Feeding VD-deficient rats with bread fermented with UV-irradiated yeast as a rich source of VD_2 increased the serum 25(OH)D dose-dependently as the concentration of VD_2 increased in the bread.[32] However, the 25(OH)D serum concentration of rats fed with VD_3 supplemented bread was higher, indicating the higher bioavailability of VD_3 containing diets. Some studies indicate that comparing to VD_2, consuming VD_3 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.[33,34] while some other studies indicate almost similar concentration of 25(OH)D with insignificant differences after ingesting either VD_3 or VD_2 on a daily basis but for a longer time (like 11 weeks).[35,36] Hence, continuously fortifying staple food products with either VD_3 or VD_2 does not necessarily or substantially affect VD bioavailability in populations.

Fortifying hens' feed with different concentrations of VD, reported promising results.[37–40] 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 VD_3 in eggs and baking wheat bread.[16] However, processing foods in the oven at regular cooking temperature for 40 min leads to a great reduction of VD_3 for about 55-61%.[16] Similar results were observed for VD_2. 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 VD_2 and VD_3 in rainbow trout fish, sunflower oil, and button mushrooms indicated the highest VD loss in pan-frying, especially for button mushrooms.[41] Interestingly, VD retention increased when lemon juice was added, which could be due to its high antioxidant activity.[42] Since VD molecules are liable to oxygen,[43] the antioxidants in lemon juice prevented oxygen degradation. During long-term storage of milk powder (for 12 months), tremendous losses of VD_3 occurred owing to thermal isomerization[44] and anti-rachitic activity found only in *cis*-triene configuration VD as a bioactive compound while other isomers like *trans*-VD and tachsyterol show little to no such activity.[45] 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.[45,46] 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,[47] whereas nano-capsules are in the range of 50-

1000 nm.[18,48] 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 3.), the efficiency depends on its size (micro or nano), utilized technique (e.g., emulsification, precipitation, coacervation, electrospinning, and liposome), coating material (e.g., whey protein, casein, hydroxylmethylcellulose) and core-to-wall ratio.[18]

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.[49] 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. [50] Under *in vitro* conditions, the bio-accessibility of VD_2 encapsulated in emulsions increased as the sizes of droplets decreased.[51] As the particle size declined, the surface-to-volume proportion increased, which resulted in accelerated interactions.[49] In contrast, *in vivo* results demonstrated that the higher the droplet size, the better the bio-accessibility of VD_2. Higher bioavailability of large emulsions containing VD_2 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 VD_2 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 VD_3, either in its free form or in β-lactoglobulin (BLG), under protracted storage, exposure to UV-C light,

and oxygen conditions indicated that VD_3 in BLG complexes (proteins sensitive to pancreatin enzymes[52]) was 3-5 times more stable than free VD_3.[53] 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 VD_3. It was proposed that the BLG-VD complex had a different assimilation pathway from that of VD alone.[53] BLG-based coagulum containing VD_3 showed even greater resistance properties against gastric destructive conditions compared with the BLG-VD complex.[54]

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 VD_3 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.[55] 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.[56,57]

Evaluating the effectiveness of high amylose starch as a nanocarrier for VD_3 through simulated gastrointestinal tests showed that the proliferation of VD_3 was slight in the gastric situation, whereas a quick initial accumulation of VD occurred in the intestinal simulant.[58] 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.[58] 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.[59]

Effect of core-to-wall ratio on VD bioavailability

oil-in-water nano-emulsion is created based on fish-oil containing VD_3 with a droplet size between 300 and 450 nm using ultrasonication technique.[60] The encapsulation efficiency was increased from 95.7% to 98.2% as the oil concentration in emulsion increased.[60] 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.[60] 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.[61] 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.

4.5. 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 VD_3 nano-emulsions *in vitro* gastrointestinal tract test revealed that the oil type significantly affected the bioavailability of VD_3.[62] 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.[62] Furthermore, long-chain triglycerides could be more appropriate carriers than medium-chain ones for VD3 delivery.[63] 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 VD_3 comparing to corn oil (digestible oil) suggested that the bio-accessibility of VD_3 remarkably declined when mineral

oil was used as a carrier under simulated gastrointestinal conditions.[64] VD_3 was inclined to maintain ensnared inside the droplets of mineral oil even throughout the protracted storage under gastrointestinal conditions. The bio-accessibility of VD_3 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 VD_3 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 VD_3'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.[65] At the other extreme, the upper level for serum 25(OH)D was considered as 225 nmol/L.[65] However, consuming more VD would result in some detrimental side effects. Besides, the upper intake level for VD_3 consumption is about 4000 IU/day (= 100 µg VD_3).[66] 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.[67] Thus, more clinical and *in vivo* studies should be carried out about different health-related aspects of using nanoencapsulation in the food industry.

Conclusions

VD_3 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.

Conflict of Interest

The authors declare they have no conflict of interest.

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Table 1. Fortification of VD in various food products

Food product	Amount of fortification	Main findings	References
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.	68
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).	69,70
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.	71
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.	72
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 VD3/serving caused no significant effects on sensory properties	73

Table 1. (*Continued*)

Food product	Amount of fortification	Main findings	References
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).	[74]
Milk	600 to 800 IU VD_3/200 mL	Daily consumption of 600 and 800 IU fortified milk fresulted 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.	[75]
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.	[76]

Table 2. Biofortification of VD in various food products

Food product	Amount of feed fortification	Period of feed fortification	Main findings	References
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 form 62.4 to 216 µg/kg feed resulted in about 7 times increase in VD of eggs.	[37]
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.	[38]
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.	[39]
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.	[40]

Table 2. (*Continued*)

Food product	Amount of feed fortification	Period of feed fortification	Main findings	References
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.	[77]
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 VD3/kg feed 2) 3000 IU VD3/kg feed 3) 1500 IU VD3 + 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.	[78]
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.	[79]
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.	[80]
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.	[81]

Table 3. Influence of food processing on vitamin D

Process Type	Food	Effect	References
Pasteurization	Milk	No significant loss	[82]
Sterilization	Milk	No significant loss	[82]
Cooking	Beef	35–42% of the original vitamin D	[83]
Roasting	Beef	Significant loss	[83]
Frying	Mushroom Egg and Margarine	Significant loss in ergocalciferol 22-24% loss in vitamin D	[41] [16]
Boiling	Egg	Significant loss in 25-Hydroxycholecalciferol 22-24% loss in vitamin D	[16,84]
Solar Drying	Fish meat Fish oil	Significant loss	[85]
Steaming	Fish meat Fish oil	Significant loss	[86]
Baking	Fish, meat Bread	Significant reduction in Cholecalciferol 24-31% loss in ergocalciferol	[84,87] [16]
Oven Drying	fish meal Smoking Fish	Significant loss Significant loss	[88] [89]

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

Encapsulation type	Wall material	*In vitro*/*in vivo*	Encapsulation efficiency (%)	Main findings	References
Microencapsulation	Medium molecular weight Manugel GHB Sodium alginate	*In vitro*	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.	[90]
Microencapsulation	chitosan/ethylcellulose complex	*In vitro*	86	Encapsulation could postpone VD_2 liberation in gastric environment, but liberate the microencapsulated vitamin in intestine environment constantly over a prolonged time.	[91]
Microencapsulation	β-lactoglobulin and lysozyme	*In vitro* and *in vivo*	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.	[92]
Microencapsulation	β-lactoglobulin	*In vitro*	-	The solubility of VD_3 was significantly increased. The capsules exhibited high resistance in the pH ranged from 1.2 to 8.	[55]
Microencapsulation	β-lactoglobulin	*In vitro* and *in vivo*	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. BLG coagula containing VD_3 might have different adsorption mechanism from free VD_3.	[53]

Table 4. (*Continued*)

Encapsulation type	Wall material	*In vitro*/*in vivo*	Encapsulation efficiency (%)	Main findings	References
Microencapsulation	Whey protein isolate (WPI) and carboxymethyl cellulose (CMC)	*In vitro* and *in vivo*	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.	93
Microencapsulation (composite gel)	Lotus root amylopectin and whey protein isolate	*In vitro* and *in vivo*	-	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.	94
Microencapsulation	Gelatin and cress seed mucilage	*In vitro and in vivo*	67.93	Ratios of mucilage to gelatin as well as core to sell 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.	61
Microencapsulation	5% WPI + 5% SPI	*In vitro*	> 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%	95
Nanocapsule	Carboxymethyl chitosan–soy protein complex	*In vitro*	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%).	59

Table 4. (*Continued*)

Encapsulation type	Wall material	*In vitro*/*in vivo*	Encapsulation efficiency (%)	Main findings	References
Nanoemulsions	Pea protein	*In vitro*	-	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.	[62]
Nanoemulsions	Quillaja saponin emulsifier	*In vitro*	-	The bioaccessibility order based on different oil carriers: medium chain triglyceride (20%) < mineral oil (40%) < orange oil (70%) < corn oil (85%) ≈ fish oil (90%).	[63]
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.	[96]
Nanoliposomes	Oleic acid and glycerol monostearate	*In vitro*	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.	[97]
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.	[98]
Pickering emulsions	Nanofibrillated cellulose (NFC)	*In vitro*	-	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	[99]

Table 4. (*Continued*)

Encapsulation type	Wall material	*In vitro*/*in vivo*	Encapsulation efficiency (%)	Main findings	References
				Cluster formation, the droplets did not coagulate.	99
Nanocomplexation	Corn protein hydrolysate	*In vitro*	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%.	100
Nanocapsule	High amylose starch	*In vitro*	37.06 to 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.	58
Nanoemulsion	WPI	*In vitro*	-	The bioaccessibility of vitamin in simulated gastrointestinal condition was remarkably higher when the oil phase was digestible than when it was indigestible.	64
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.	101
Nanoemulsions	Fish oil	*In vitro*	95.7–98.2	The nanoemulsions had a shelf life of > 90 days. The nanoemulsions had antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. The nanoemulsions displayed higher stability under gastrointestinal condition.	60
Nanocomplex	Ovalbumin and high methoxyl pectin	*In vitro*	96.37	Up to 80% VD release was occurred in intestinal fluid.	17
Nanoemulsion	Tween 80 and Soy lecithin	*In vitro*	-	Increasing storage temperature (4 vs 25 ˚C) decreased VD retention (74.4 vs 55.3) after 1 month.	102

Table 4. (*Continued*)

Encapsulation type	Wall material	*In vitro*/*in vivo*	Encapsulation efficiency (%)	Main findings	References
Nanoemulsion	Pea protein isolate	*In vivo*	-	The VD deficient rats treated with nanoencapsulated VD had normal serum 25(OH)D level.	[103]
Nanoemulsion	Alginate and chitosan	*In vitro*	-	Co-encapsulation of VD_3 and calcium decreased the bioavailability of VD.	[104]
Nanoemulsion	Pea protein	*In vitro*	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.	[50]

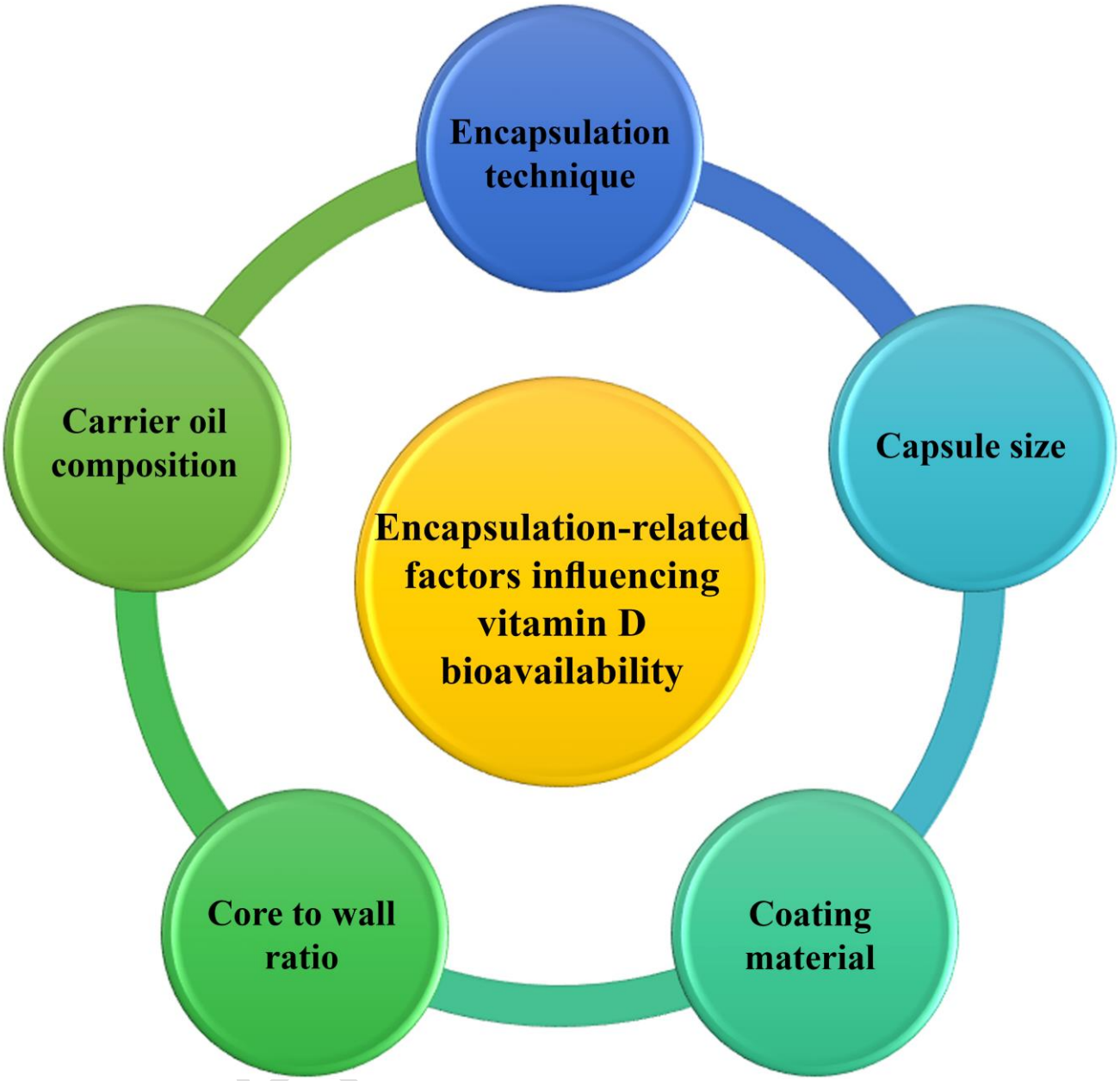

Figure 1. The main encapsulation parameters which are affecting the bioavailability of vitamin D.

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