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Food Chemistry



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Advancing food fortification with kinetics: Stabilizing vitamin D₃ through calcium carbonate-based vehicles



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ARTICLE INFO

Keywords: Vitamin D Calcium carbonate Vitamin E derivative Food vehicles Kinetics

ABSTRACT

The addition of vitamin D_3 in food products is an important health strategy. The vitamin D is heat, moisture, oxidation and pH sensitive. Given the complexity and diversity of food matrices, the use of various encapsulation strategies can more effectively address the need for vehicle system, that ensure the long-term chemical and physical stability of vitamin D. Calcium carbonate-based vehicle, modified with polyvinyl alcohol (PVA) and D α -tocopherol succinate, were synthesized and loaded with vitamin D₃ (D₃/X/CaCO₃). The vehicle has a form of beadlet designed to protect vitamin D₃. The obtained nanostructured D₃/X/CaCO₃ demonstrated the desirable characteristics for powdered food matrix-vitamin D based, such as: enhanced thermal stability of vitamin D, efficient loading, and an extended shelf-life. A detailed analysis of the physicochemical behavior of vitamin D, both structurally and kinetically, was provided presenting the fully functionalized pre-food model systems. The proposed kinetic perspective could serve as a roadmap for vitamin D₃ stability.

1. Introduction

Food fortification is known as a process in which some micronutrients, such as vitamins, minerals, or bioactive compounds, are added to food. Four decades ago, the Food and Drug Administration (FDA) established the Food Fortification Policy, guided by 6 fundamental principles (Dwyer et al., 2015). Food fortification is considered to be a very important public health strategy. Various approaches exist for food enrichment, with encapsulation being one of the most prominent methods (Miano & Rojas, 2023). Given the complexity and variety of food structures, utilizing different encapsulation strategies could more effectively meet the need to develop vehicle systems that ensure long-term chemical and physical stability. The encapsulation process is particularly important for certain micronutrients that are prone to instability. In particular, the sensitivity of vitamins is a major concern. The vitamins, in general, show light, pH, heat, oxidation, and hydrolysis instability (Bajaj et al., 2021). Vitamin D, as a representative of the liposoluble vitamins, is such an example. Even though it is considered as a vitamin, it is actually a prehormone. The main role of vitamin D is to regulate calcium and phosphate homeostasis, but it is increasingly gaining attention due to its association with several serious modern health issues. Under the term vitamin D, both D₂ (ergocalciferol) and D₃ (cholecalciferol) are considered. Even though there are many natural sources of vitamin D, such as salmon, eggs, fish liver oil, beef, etc., the worldwide population faces a vitamin D deficiency (Dominguez et al., 2021; El Hoss et al., 2023; Hewavitharana & Gomes, 2016). Fortification of food with vitamin D is very common in dietary products. Also, by the addition of vitamin D into dairy products, the situation concerning childhood rickets was quite improved (Goebel et al., 2021; Welch et al., 2000). The low intake of vitamin D fortified milk was found to be in connection with vitamin D deficiency (Itkonen et al., 2021, Keane et al., 1998, Kinyamu et al., 1998). There is evidence that aggravation of

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https://doi.org/10.1016/j.foodchem.2025.144811

Received 26 March 2025; Received in revised form 13 May 2025; Accepted 16 May 2025 Available online 17 May 2025

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vitamin D deficiency could be connected with low calcium intake. Milk products are fortified with calcium salts, such as citrate, phosphate, carbonate and lactate. However, one should be very careful since calcium salts can destabilize milk proteins (Cox & Miller, 2002). Vyas et al. showed that calcium salts influence pH of milk, which is in connection with stability and solubility for skim powdered milk (Vyas & Tong, 2004). For instance, lactate and phosphate ions reduce the pH value, while carbonate ions increase the pH. Calcium carbonate proved to be the most suitable salt when it comes to heat stability maintenance. It contains about 40 % of calcium content and solubility of around 0.153 mmol/L. Calcium presence can mask taste, improving organoleptic properties of products and stability (Bostan & Ghaitaranpour, 2019; Sedighi et al., 2025).

In case of powdered food, vitamin D3 should be protected and encapsulated into/onto some proper vehicle. Moreover, the unprotected, free form of vitamin D exhibits several drawbacks, including reduced stability, rapid release, and non-homogeneous dispersion within the food matrix (Beauchet et al., 2019). Innovative approaches aimed at minimizing vitamin D degradation and enhancing its stability increasingly favor the use of specialized micro- and nano-structured delivery systems (El Hoss et al., 2023; Lavelli, 2021). Xiang et al. used a protein-polysaccharide nanocomplex consisting of ovalbumin and methoxyl pectin to protect vitamin D from acidic environment, as well as to control its release (Xiang et al., 2020). Abasi et al. showed that if a protein isolates nanoparticles, containing different calcium concentration, this leads toward enhanced stability of vitamin D. It was shown that protein-isolated nanoparticles delay the degradation of vitamin D. Also, the presence of calcium enhances the structural stability of formulation, inhibiting the oxygen diffusion into particle (Abbasi et al., 2014). Furthermore, an encapsulation of vitamin D in amylose complex proved to enhance stability (both thermal and light) and also release of vitamin D. The thermal stability of vitamin D was increased by 29 % and photostability around 59 % after encapsulation of vitamin D in amylose inclusion complex (Liu et al., 2023).

Therefore, we have synthesized calcium carbonate-based vehicles for improved vitamin D loading and stability. Vitamin D, cholecalciferol, was encapsulated into/onto calcium carbonate-based vehicles using adsorption (ADS) and coprecipitation (COPR) approaches: $D_3/X/CaCO_3$, where X is polyvinyl alcohol, PVA ($D_3/PVA/CaCO_3$)_{ADS} or D- α -tocopherol succinate (vitamin E derivative) ($D_3/vitE/CaCO_3$)_{COPR}.

The polymeric component can enhance stability during the encapsulation process. For instance, for encapsulation and stabilization of vitamin A in the form of microparticles, methacrylate copolymer was used (Tang et al., 2022). Methacrylate copolymer is thermostable, pHsensitive, and FDA-approved. It was shown that such an approach results in improved stability during long-term storage and cooking. Zapelini de Melo reported on the encapsulation of vitamin D with zein biopolymer, which gave favorable properties for gastrointestinal and food processing (Zapelini de Melo et al., 2021). Tchuenbou-Magaia et al. reported the development of chitosan/zein microparticles coencapsulating vitamin D3 and rutin, proposing this system as a suitable and scalable approach for food fortification. Within the formulation, rutin served as a stabilizing agent. Both vitamin D3 and rutin were retained in their amorphous states, which contributed to improved bioavailability (Jelić et al., 2024; Tchuenbou-Magaia et al., 2022). Luo et al. prepared and characterized also zein/chitosan complex for the encapsulation of α -tocopherol. It was concluded that the encapsulation of α -tocopherol within polymer-based delivery systems can not only enhance its stability but also improve its bioavailability and enable controlled release in vivo (Luo et al., 2011). It has been confirmed that the sensitivity of vitamin D to humidity, oxygen, and interactions with minerals can be mitigated by certain antioxidants, such as tocopherol (vitamin E), thereby enhancing the stability and preservation of cholecalciferol (Tang et al., 2022).

For the sake of comparison and potentially changing and enhancing features of calcium carbonate-based vehicles, we have synthesized two control vehicles consisting only of vitamin D and bare calcium carbonate: $(D_3/CaCO_3)_{ADS}$ and $(D_3/CaCO_3)_{COPR}$.

Although fortification strategies are well-established, there is still a lack of kinetic data integration, which limits predictive modeling under industrial conditions (Lavelli et al., 2021; Peleg et al., 2017; van Boekel, 2008). The absence of kinetic data hinders the ability to predict the stability of vitamin D under industrial processing conditions. We hypothesized here that by encapsulating vitamin D₃ into/onto the proper vehicle and knowing the structure and kinetics in detail, thermal stability and long-term storage in the food fortification process can be enhanced. This research offers examples of vitamin D₃ and Ca²⁺- rich vehicle systems with a mechanistic explanation of vitamin D physicochemical behavior in order to obtain models of fully (structurally and kinetically) functionalized pre-food systems. We believe that an understanding of the kinetic parameters, such as activation energy, preexponential factor A, constant rate, etc., could allow us to predict the behavior of vitamin D during the fortification process and beyond. The literature survey showed insufficient implementation of kinetics data in the food industry, even though such data can be used as a basis to design the best formulation to deliver vitamin D₃ with functional food. Recently, Yang et al. explored the stability of vitamins (A, B₁, and C) in different enteral feeding formulas during storage at different temperatures from a kinetic point of view (Yang et al., 2024).

The findings are expected to help bridge the gap between kinetic studies and their practical implementation in the food industry, acknowledging the complexity of chemical and physical interactions within food matrices. Specifically, the study provides a kinetic framework for the design of effective and functional food ingredient systems. The proposed computational kinetics approach holds potential to advance the field of food science by enabling more precise modeling of key quality attributes, such as shelf-life prediction, food preservation and by supporting the optimization of food processing parameters.

2. Materials and methods

2.1. Synthesis of systems

Reagents: CaCl₂, Sigma-Aldrich, CAS: 10043–52-4, Na₂CO₃, Sigma-Aldrich CAS: 9002-89-5, C₂₇H₃₃O, DSM, USA, CAS: 67–97-1, D- α -toco-ferol succinate (vitamin E derivate), C₃₃H₅₄O₅, Sigma-Aldrich, CAS 4345-03-3.

Calcium carbonate-based vehicles were synthesized using equimolar solutions of Na₂CO₃ and CaCl₂. The pH of the initial solutions of Na₂CO₃ and CaCl₂ was 8 and 12, respectively. pH values were adjusted using 0.1 mol/L HCl and 0.1 mol/L NaOH solutions. The as-prepared solutions were mixed on a magnetic stirrer (500 rpm for 1 h). After synthesis, all specimens were separated from the solution by centrifugation and then washed three times with distilled water and ethanol to remove any impurities. Drying was performed at t = 70 °C for t = 1 h.

For the adsorption-based approach $((D_3/CaCO_3)_{ADS}; (D_3/PVA/CaCO_3)_{ADS})$, 25 mL of ethanolic solution of vitamin D_3 (50 mg mL-1) was poured onto the prepared calcium carbonate vehicle. The procedure for the modified vehicle, PVA/CaCO₃, was published previously (Janković et al., 2023).

In case of the coprecipitation mechanism $((D_3/CaCO_3)_{COPR}; (D_3/vit E/CaCO_3)_{COPR})$, vitamin D_3 (50 mg) and D- α -tocoferol succinate (vitamin E derivate) (20 mg) were mixed together with solutions for calcium carbonate preparation.

The solutions for adsorption and coprecipitation approaches were mixed on a magnetic stirrer for 3 h at 500 rpm, after which the solutions were centrifuged, and the powder was washed three times with distilled water. The samples were dried at room temperature and stored in a dark place. They were also kept in the freezer prior to characterization.

2.2. Characterization of synthesized materials

2.2.1. The X-ray powder diffraction (XRPD) analysis

The X-ray diffraction measurements were performed by the Rigaku MiniFlex diffractometer (Rigaku Corporation, 3–9-12, Matsubara-cho Akishima-shi, Tokyo 196–8666, Japan), using Cu-K α radiation (λ = 0.15418 nm) operated with a tube voltage of 40 kV and a tube current of 15 mA, and equipped with a secondary monochromator (graphite). The step method was applied (step size of 0.02°) in the 20 range of 3° - 60°. Crystallite size (*D*) was calculated using Scherrer's formula after correcting for the instrumental broadening.

2.2.2. Thermal analysis (simultaneous TG-DTG measurements)

Thermal stability of the investigated samples was tested using a thermogravimetric analyzer manufactured by TA instruments (159 Lukens Dr., New Castle, DE 19720, USA) with the device model SDT 2960, which is capable for the simultaneous thermogravimetry (TG) – derivative thermogravimetry (DTG) – differential thermal analysis (DTA) measurements. Thermal analysis experiments were performed using platinum crucibles, in the temperature range from the room temperature (RT) up to T = 800 °C, under a dynamic nitrogen (N₂) atmosphere (nitrogen (N₂) purity of 99.999 wt%) at a flow rate of $\varphi = 100$ mL/min. The tested powder samples with the mass about $\Delta m = 5.0$ mg were placed in platinum crucibles at the heating rate of 10 °C/min. At a given heating rate, recording for each sample was performed twice due to the coexistence of data and its reproducibility. The TGA data were used as input data for the kinetic software Kinetics2015.

2.2.2.1. *Kinetic analysis.* The mass data obtained by TGA were transformed into conversion degree (a) using the following equation:

$$\alpha = \frac{m_o - m_t}{m_o - m_f} \tag{1}$$

where m_t represents the mass of the sample at arbitrary time t, whereas m_0 and m_f are the mass of the sample at the beginning and at the end of the process, respectively. Such data are easily used by proper kinetics software which in return provide determination of corresponding kinetic triplet: E_{α} , A and $f(\alpha)$, where A is the pre-exponential factor, E_{α} is the apparent activation energy, and $f(\alpha)$ is a mathematical expression of kinetics mechanism (Vyazovkin et al., 2020). The exact determination of kinetic parameters is based on multiple scan methods, which require the measurements at different heating rates (non-isothermal), and use the data sampled at common conversion degrees (isoconversion, model-free methods). Non-isothermal regime is highly recommended by the International Confederation for Thermal Analysis and Calorimetry (ICTAC) (Koga et al., 2023). Once the E_{α} is determined, it is possible to search for the function (kinetic model, $f(\alpha)$) that enables the best fit of experimental data. Kinetics software Kinetics2015 (GeoIsoChem, Covina, CA, USA), used in this study, summarizes linear and nonlinear regression analysis methods. All the linear regression methods available in this software are variations of isoconversional methods (Kinetic software KINETICS2015). To check the presumed invariance of E_{α} on conversion degree, the software Kinetics15 performs the isoconversional "modelfree"analysis by means of the expanded Friedman method (Burnham & Dinh, 2007), which is based on the logarithm of Eq. (2):

$$\ln\left(\beta\frac{d\alpha}{dT_{\alpha}}\right) = -\left(\frac{E_{\alpha}}{RT_{\alpha}}\right) + \ln[A_{\alpha}(1-\alpha)]$$
⁽²⁾

The ratio $-E_{\alpha}/R$ is the slope and the $\ln\{A_{\alpha}(1-\alpha)\}\$ may be determined as the intercept with the vertical axis of the plot of $\ln(\alpha d(1-\alpha)/dT_{\alpha})$ vs. 1/ T_{α} . The expanded Friedman method can be applied to any thermal history. Also, kinetic software includes majority of reaction models, ranging from 1st order, nth order, nucleation-growth model, as well as the activation energy distribution models, and uses a nonlinear regression methods to determine the parameters of the Eq. (2) to fit in a best way the experimental data (Vyazovkin & Wight, 1999). The TGA results were used as the input data in order to obtain kinetic parameters: activation energy and pre-exponential factor A.

2.2.3. MALDI (matrix-assisted laser desorption/ionization) – Mass spectrometry (MS) analysis

All mass spectra in this study were recorded by a matrix-assisted laser desorption/ionization time of flight mass spectrometer MALDI-TOF-MS (Voyager-DE PRO, Sciex, USA) equipped with a nitrogen pulse laser source (the wavelength of 337 nm, pulse duration of 3 ns, repetition rate of 20 Hz). These mass spectra were obtained by accelerating voltage of 25 kV, grid voltage of 90 %, delayed extraction time of 100 ns, and the laser intensities of 3200 arbitrary units, 200 laser shots per spectrum in reflection positive ion mode. The stoichiometric composition of each detected ion was obtained by comparing its experimental and theoretical isotopic distributions. Further in the text, for simplicity, the mass-to-charge ratio (m/z) corresponding to the most intense peak in the series of peaks is listed.

As matrix 2,5-dihydroxybenzoic acid ((HO)₂C₆H₃CO₂H – DHB, 154.12 g mol⁻¹) was used. This matrix was purchased from Sigma-Aldrich (Markham, Ontario, Canada). 10 mg/mL DHB in trifluoroacetic acid-water-acetonitrile (0.1,50:50, $\nu/\nu/\nu$) solution was prepared.

Samples were prepared as follows: 9.80 mg of the vitamin $D_3/CaCO_3$ sample was dissolved in 100 μL acetic acid (ReagentPlus®, \geq 99 %, purchased from Sigma-Aldrich Chemie GmbH); 10.27 mg of the vitamin $D_3/vitE/CaCO_3$ sample was dissolved in 100 μL ethyl acetate (ReagentPlus®, \geq 99 %, purchased from Sigma-Aldrich Chemie GmbH). After the first dissolution, 7.5 μL of acetic acid was added to the solution.

The samples of 1 μ L were placed on a MALDI 201 stainless steel plate and dried in air. After that, 1 μ L of the matrix solution was applied to the samples and air dried at room temperature before the stainless steel plate was inserted into the device (Patil et al., 2024).

2.2.4. Atomic force microscope, AFM

AFM images were measured at several points of the investigated specimens in phase mode on a SPM 9700 HT (Shimazu Co.) using an AFM tip with the nominal resonance frequency of 320 kHz and a force constant of 42 N/m. The measurements were done with a scan rate of 2 Hz, while the resolution of the obtained images was 512 lines per scan direction. Raw data were just flattened and analyzed using the software package Gwyddion. Flattening was done by fitting with a polynomial function of the form:

$$P(x,y) = \sum_{j=0}^{m} \sum_{k=0}^{n} a_{j,k} x^{j} y^{k}$$
(3)

where n and m denote the horizontal and vertical polynomial degrees, respectively, and subsequent subtraction of the corresponding background representation.

The samples (D₃/CaCO₃)_{ADS}, (D₃/CaCO₃)_{COPR} and (D₃/vitE/CaCO₃)_{COPR} were dispersed in water and treated for ~30 min in an ultrasound bath. In case of the samples (D₃/PVA/CaCO₃)_{ADS}, acetonitrile was used as a dispersion medium. A drop of each prepared dispersion system was put on a glass substrate with dimensions 7 mm × 7 mm. After evaporation of dispersion medium, the specimens, consisting of dispersed nanoparticles, were used for AFM measurements.

3. Results and discussions

3.1. Characterization of calcium carbonate-based vehicles loaded with vitamin $\ensuremath{D_3}$

3.1.1. AFM and XRPD analysis

2D and 3D AFM height images (Fig. 1- a1, b1, c1, d1, a2, b2, c2 and d2) confirm the nanostructured character of studied specimens. Nano- or micro- structured matrix used for vitamin D delivery in the fortification



Fig. 1. AFM height (2D and 3D) and phase images of: (D₃/CaCO₃)_{ADS} (a), (D₃/CaCO₃)_{COPR} (b), (D₃/vitE/CaCO₃)_{COPR} (c) and (D₃/PVA/CaCO₃)_{ADS} (d).

process is considered to be an effective and promising strategy due to a significantly larger available surface area with tailored surface morphology (Hethnawi et al., 2023; Lavelli, 2021). Hethnawi et al. showed that the use of starch-based nanoparticles surface-decorated with vitamin D₃ offers a promising strategy for enhancing vitamin D₃ stability and bioavailability in functional food or nutraceutical applications. The formulated system enabled a diffusion-controlled release profile of vitamin D₃, in contrast to its direct administration (Hethnawi et al., 2023). Silva et al. reported on improved stability of 70 % for vitamin D when encapsulated in nanoemulsions, compared to emulsion (Silva et al., 2012). Also, nano-vehicle in the form of corn protein hydrolysate enhanced the stability and bioavailability of vitamin D (Lin et al., 2016). The AFM The D₃/CaCO₃ nanoparticles (NPs) prepared using a coprecipitation approach are, on average, smaller compared with corresponding NPs obtained by adsorption (Fig. 1-a1, b1, a2 and b2). Assuming that CaCO₃ NPs have similar dimensions, the D₃ vitamin layer is thicker in the case of the adsorption approach. The loading of vitamin D₃ on bare CaCO₃ NPs results in the presence of more than one phase in AFM phase images (Figs. 1-a3 and b3), implying that some of NPs' surfaces are not completely covered by vitamin D₃. This effect is more pronounced in the specimen obtained by the coprecipitation method, which, notably, aligns with the presence of a thinner layer of adsorbed vitamin D₃. The most effective covering of NPs' surfaces is achieved in the case of modified CaCO3-based vehicles with PVA (Fig. 1d3), while the least coverage was for CaCO3 surface modified using vitamin E (Figure 1c3). In addition, CaCO₃ NPs modified by vitamin E have a more pronounced tendency toward coalescence (Figure 1 c2).

The better coverage of NPs' surfaces by D_3 vitamin in the case of surface-modified CaCO3, obtained by adsorption approach, in comparison to the corresponding bare CaCO₃, can be ascribed to the stronger interaction between the modifying layer and loaded D_3 . Generally, vitamin D interacts due to its open B-ring and flexible side chain. Also, it possesses exocyclic covalent double bonds (Martínez-Núñez, E., Cabaleiro-Lago, E.M., Fernández-Ramos, A., Hermida-Ramón, J.M., Peña-Gallego, A., 1999). The results from AFM measurements imply that the chemical identity of the modifying layer is of great importance, influencing the stability of the obtained specimens. The most effective coverage with D₃ vitamin, achieved using PVA as a modifying layer, is due to the favorable interaction between the polymeric layer and D3 and good adhesion of the polymeric shell on the CaCO₃ NPs' surfaces as well (Labidi & Djebaili, 2008).

The structure of modified vehicles (D₃/vit E/CaCO₃)_{COPR} was further confirmed by XRD analysis. The XRD of (D₃/PVA/CaCO₃)_{ADS} was previously published, revealing the combination of crystalline and amorphous (20 %) nature of the formulation (Janković et al., 2023). Fig. 2 presents the XRD pattern of (D₃/vit E/CaCO₃)_{COPR}, which reveals the presence of CaCO₃ (JCPDS card: 83–0578). The calcite structure has a rhombohedral structure with a = b = 4.9 Å, and c = 17.06 Å with a space group R-3c (Hayakawa et al., 2008). The second identified phase according to reflections is provided by Wang et al. (Wang et al., 2016). Wang et al. identified observed reflections as a crystalline orthorhombic



Fig. 2. XRD pattern of a D- α -tocopherol succinate supported calcitecholecalciferol. The Miller indices correspond to the ones of the CaCO₃ phase found from JCPDS 83–0578. Cholecalciferol reflections are marked with stars. The D- α -tocopherol reflection is marked with the cross.

solid-state form of cholecalciferol (form A, two conformers α/β , 1:1). It was reported that the α conformer is more eligible for photo- or thermoinduced reactions then conformer β . The sharp peaks at the following 2θ values: 5.1°, 6.7°, 13.7°, 15.7°, 18.1°, and 21.8° in XRD patterns (Fig. 2), are due to cholecalciferol (Nayak et al., 2019). In research by Naghibzadeh et al. (Naghibzadeh et al., 2010) and Ali Raza et al. (Raza et al., 2020), it is determined that pure α -tocopherol gives a reflection at $2\theta \sim$ 19°, while Walejko et al. (Wałejko et al., 2019) reported on intensive peak for tocopherol succinate at $2\theta = 16.2^{\circ}$. In our research, the observed reflections are of low intensity, likely due to the small quantity of D- α -tocopherol succinate.

3.1.1.1. Crystallinity of calcium carbonate-based vehicle. The crystallite size of CaCO3 was calculated using Scherrer's equation. The average calcite crystallite size of the examined sample was $39.12 (\pm 6.0)$. The crystallite size and lattice strain using the Williamson-Hall plot are estimated to be 75.76 nm and 0.00127, respectively. The CaCO₃ crystallite size determined by the Scherrer equation and Williamson-Hall plot is found to be different. A present microstrain most definitely causes this. As previously determined by (Kim et al., 2014) Scherrer equation cannot be employed to assess particle sizes when lattice strain is present (this is usually the case when crystals are co-precipitated with additives). This implies that the crystallite size of around 75 nm is more accurate. In previous research (Janković et al., 2023), the presence of PVA and different synthesis methods resulted in a CaCO₃ average crystallite size of 30.85 nm (Scherrer analysis) and 32.93 nm (Williamson-Hall analysis). In this case, the determined low microstrain value of 0.00031676 presumably originates from surface and core defects.

3.1.1.2. Crystallinity of cholecalciferol. The crystallite size of coprecipitated cholecalciferol is also determined by the Scherrer equation and by the Williamson-Hall plot. Calculations were performed based on the four most intense peaks. The particle size determined is found to be highly uncertain and variable, with an average size of 56.03 ± 29 nm. Williamson-Hall plot indicates a strong microstrain value present 0.00294 and crystalline size 96.96 nm. In previous research by Haham et al. (Haham et al., 2012) on the stability and bioavailability of vitamin D nanoencapsulated in casein micelles, it was determined that the average volume-weighted diameters of particles (aggregates) were about 90–95 nm. This is almost the same as the crystallite value determined by the Williamson-Hall plot.

It is worth mentioning that previous research on the VD₃/PVA/ CaCO₃ nanocomposite scaffold (Janković et al., 2023) revealed significantly different results. Diffraction peaks of D₃ were not visible in the XRD pattern, indicating an amorphous rather than crystalline compound.

The food powder can exist in both crystalline and amorphous forms, or a mixed one, and the structure affects stability and applicability to a large extent. The proportion and structure of amorphous and crystalline phases play a critical role in both storage conditions and production processes. A key challenge associated with the amorphous structure is its inherent instability. Consequently, it is essential to develop a formulation/carrier that ensures the stability of the amorphous form (Bhandari & Howes, 1999). This is particularly significant as the amorphous phase exhibits higher solubility compared to the crystalline phase, a property that is especially valuable when dealing with poorly soluble compounds, such as lipophilic vitamin D (Janković et al., 2023; Jelić et al., 2024). An amorphous structure is sometimes desirable, for example, in the preparation of fruit-based products or milkshakes. Amorphous icing sugar dissolves more quickly and efficiently than crystalline sugar (Chen & Chou, 1993). Similarly, amorphous silica is used as a food additive (E551), which has been approved by the FDA (Merget et al., 2002).

3.1.2. MALDI-MS analysis

The MALDI mass spectrum of $(D_3/CaCO_3)_{ADS}$ in the positive mode

showed that the Ca(II) ion is bound by a D₃ molecule, as the ions [D₃Ca (CH₃COO)(OH) + 2H₂O]⁺ and [D₃Ca(CH₃COO)₂ + nH₂O] + (*n* = 2, 3) were detected (Janković et al., 2023). Characteristic ions of vitamin D₃ (C₂₇H₄₄O) were also detected in this spectrum (*m*/*z* 385 corresponds to a protonated molecular ion, and *m*/*z* 367 corresponds to a protonated molecular ion without a water molecule). Similar to the previous case, the MALDI mass spectrum of (D₃/CaCO₃)_{COPR} in the positive mode (Fig. 3a) contains the ion [D₃Ca(CH₃COO)₂ + 2H₂O]⁺ (*m*/*z* 578.08, calcd 578.35). It should be emphasized that the ions [D₃Ca(CH₃COO) (OH) + 2H₂O]⁺ and [D₃Ca(CH₃COO)₂ + 3H₂O]⁺ were not detected. However, additional peaks were detected at *m*/*z* 407.87, 424.87 and 439.88 corresponding to [D₃Ca-CH₄]⁺ (calcd 408.27), [D₃Ca]⁺ (calcd 424.31) and [D₃CaO]⁺ (calcd 440.29), respectively, which had not been previously identified. In all cases, the ligand CH₃COOH originated from the solvent used.

The MALDI mass spectrum of $(D_3/PVA/CaCO_3)_{ADS}$ showed that PVA positively influences the interaction of calcium with the molecules of vitamin D_3 , i.e. in this case the Ca(II) ion is bounded by two D_3 molecules in the ions $[2D_3Ca(OH)_2(CH_3COO)_2]^+$, $[2D_3Ca(CH_3COOH)_2 + 3H_2O]^+$ (Janković et al., 2023).

The selected part of the MALDI mass spectrum of $(D_3/vitE/CaCO_3)_{COPR}$ in positive mode is shown in Fig. 3b. In addition to the vitamin $[D_3]^+$ ion, this mass spectrum contains a typical ion of vitamin E $(C_{29}H_{50}O_2)$ at m/z 430.55 (calcd 430.71), which is consistent with previous results in the literature (Jiang et al., 2015). A low intensity peak at m/z 854.72 (calcd 854.68), identified as $[D_3-Ca-vitE]^+$ ion, was also detected in this spectrum, which may indicate that the presence of calcium ensures the interaction between D_3 and vitamin E.

3.1.3. TG/DTG analysis

3.1.3.1. Thermal stability of calcium carbonate-based vehicle loaded with vitamin D. Fig. 4 presents the simultaneous TG/DTG curves for pure vitamin D (Fig. 4a), (D₃/CaCO₃)_{ADS} (Fig. 4b), (D₃/PVA/CaCO₃)_{ADS} (Fig. 4c), (D₃/CaCO₃)_{COPR} (Fig. 4d) and (D₃/vit E/CaCO₃)_{COPR} (Fig. 4e) systems. Thermal decomposition of pure vitamin D is a single-step process, while other systems investigated are multistep processes. (D₃/ CaCO₃)_{ADS} and (D₃/CaCO₃)_{COPR} thermally decompose in three steps (I dehydration process; II - decomposition of vitamin D and III - decomposition of calcium carbonate). Thermal decomposition of (D₃/PVA/ CaCO3)ADS is a four step process (I - dehydration process; II - decomposition of vitamin D; III - decomposition of PVA and IV - decomposition of calcium carbonate) and (D₃/vit E/CaCO₃)_{COPR} also decompose in four stages (I - dehydration process; II - decomposition of vitamin D; III decomposition of D-a-tocopherol succinate and IV - decomposition of calcium carbonate). I stage in all systems, except for pure vitamin D, is the dehydration process, in which mass loss corresponds to water loss (moisture). For $(D_3/CaCO_3)_{ADS}$, $(D_3/CaCO_3)_{COPR}$ and $(D_3/vit E/CaCO_3)_{COPR}$ CaCO₃)_{COPR} the moisture loss is approximately 3 %, and for the (D₃/ PVA/CaCO₃)_{ADS} it is \sim 5 %. The water loss is presented with a small, rather diffuse peak. The moisture content is very important for the stability of food powder, since it can start the process of recrystallization of amorphous parts, but it can also affect the flowability of food powder (Amstrong et al., 2014). On the TG curve of pure vitamin D (Fig. 4a), we can see that thermal decomposition of vitamin D starts at 146 °C and ends at 310 °C. The highest mass change is at 240 °C. Tsai et al., using DSC analysis, reported 166 °C and 156 °C as decomposition temperatures of D_2 and D_3 , respectively (Tsai et al., 2017). Even though the decomposition takes place as a single-stage process, it is a very complex one. Vitamin D is a rather large sterol molecule, lipophilic in nature, with susceptibility to oxidative or heat degradation (Lavelli, 2021). When it comes to heat, a conversion reaction vitamin $D_3 \leftrightarrow pre$ -vitamin D3 occurs (Meana-Pañeda & Fernández-Ramos, 2012). Mass spectrometry of vitamin D studies showed the existence of two significant fragments: m/z 385 (protonated molecular ion) and m/z 367 (protonated



Fig. 3. a (up) and 3b (down). The MALDI-MS spectrum of (D₃/CaCO₃)_{COPR} and MALDI-MS spectrum of (D₃/vit E/CaCO₃)_{COPR}.

molecular ion without one water molecule) (Mahmoodani et al., 2018). We have observed the same. Also, cis/trans conversion could take place (Mahmoodani et al., 2018). It is important to emphasize that cis-triene configuration is known for its biological activity (anti-rachitic activity), while some isomers, such as the trans isomer of pre-vitamin D (i.e., tachysterol), have no or very little activity (Mahmoodani et al., 2018). Temperature or low pH can also isomerize vitamin D to isotachysterol via tachysterol and pre-vitamin D₃ (Hanewald et al., 1968; Jin et al., 2004). The air atmosphere can give oxidation products such as 1-hydroxy-vitamin D3, 1-keto-vitamin D3, pyrocalciferol, and isopyrocalciferol (Jelić et al., 2023). It is noteworthy that the DSC curves for the degradation of UV-irradiated vitamin D3 showed no significant differences in O₂ and N₂ atmosphere (Tsai et al., 2017). Taking into account all the parameters that could contribute to instability, keeping vitamin D stable (especially thermally stable) during the fortification process is a challenging task.

The thermal stability of vitamin D₃ loaded on both unmodified and modified salt carriers is as follows: for unmodified vehicles, the thermal decomposition of D₃ occurs within the range of 184 °C–310 °C for (D₃/ CaCO₃)_{ADS} and 155 °C–310 °C for (D₃/CaCO₃)_{COPR}. In contrast, the degradation of D₃ in modified vehicles exhibits enhanced thermal stability, occurring at 199 °C–400 °C for (D₃/vitE/CaCO₃)_{COPR} and 206 °C–340 °C for (D₃/PVA/CaCO₃)_{ADS}. The thermally induced decomposition process of vitamin D₃ clearly demonstrates an improvement in stability, particularly in modified vehicles. Adsorption appears to delay the thermal degradation of vitamin D₃ by approximately 60 °C, whereas coprecipitation results in a lower shift, around 50 °C. This delayed thermal decomposition strongly supports the enhanced thermal stability of vitamin D₃ in modified carriers. Following the decomposition temperature, thermal stability of vitamin D is given in the following manner: D₃ < (D₃/CaCO₃)_{COPR} < (D₃/CaCO₃)_{ADS} < (D₃/vitE/CaCO₃)-_{COPR} < (D₃/PVA/CaCO₃)_{ADS}.

The vitamin D peak (Fig. 4a) is well-defined and sharp. However, its intensity varies depending on the vehicle system. Notably, for modified calcium carbonate vehicles, the vitamin D peak is significantly higher compared to unmodified calcium carbonate vehicles, particularly in the case of (D₃/CaCO₃)_{ADS}. These observations suggest that the calcium carbonate vehicle exhibits a capacity-dependent ability to incorporate and retain vitamin D within or onto its structure. The amorphous formulations typically exhibit a higher capacity for adsorbing external molecules (Ho et al., 2017). Vitamin D is considered to be a highly flexible molecule and has the ability to adopt different geometrical structures. We have evaluated entrapment efficiency (EE%) of vitamin D using TGA curves (Liang et al., 2021). The EE% are as follows: (D_3 / CaCO₃)_{ADS}-18.5 %; (D₃/CaCO₃)_{COPR}-36 %; (D₃/vitE/CaCO₃)_{COPR}-50.5 % and (D₃/PVA/CaCO₃)_{ADS}-51.0 %. Further observations can be made by comparing the results obtained using the AFM and MALDI methods. Based on TG/DTG/AFM/MALDI analyses, the highest coverage was achieved with (D₃/PVA/CaCO₃)_{ADS}. This can be attributed to three key factors: (i) the use of an adsorption-based approach, (ii) the combination of calcium carbonate and PVA, which resulted in a geometric structure highly favorable for incorporating vitamin D molecules due to calcium's complexation ability, and (iii) the capacity of PVA to attract and accommodate multiple vitamin D molecules. For the (D₃/VitE/ CaCO₃)_{COPR} system, the AFM study confirmed the presence of an ultrathin layer, whereas the TG/DTG analysis indicated a relatively high binding efficiency of vitamin D based on EE%. These findings are entirely consistent, as AFM primarily assesses surface characteristics (Hethnavi et al., 2023). Notably, MALDI analysis revealed a rather unexpected interaction between vitamin D and vitamin E, forming the [D3-



Fig. 4. The simultaneous TG/DTG curves for pure vitamin D (Fig. 4a), (D₃/CaCO₃)_{ADS} (Fig. 4b), (D₃/PVA/CaCO₃)_{ADS} (Fig. 4c), (D₃/CaCO₃)_{COPR} (Fig. 4d) and (D₃/vit E/CaCO₃)_{COPR} (Fig. 4e) systems.

Ca-VitE] + complex, despite their hydrophobic nature. This suggests that the calcium ion may have facilitated this interaction. The symmetrical DTG peak associated with vitamin D exhibits strong intensity, nearly overlapping with the DTG peak of D- α -tocopherol succinate. The thermal decomposition of D- α -tocopherol succinate begins at 181 °C in a nitrogen atmosphere (Ushikusa et al., 1991). After a 25 % mass loss, the decomposition behavior of D-α-tocopherol succinate aligns with that of D- α -tocopherol. Initially, the succinate moiety is released, followed by the thermal degradation of D- α -tocopherol. Li et al. reported on the existence of calcium succinate complex, demonstrating notable thermal stability (Li et al., 2025). The thermal stability of tocopherols was found to be dependent on the nature of the esterified moiety. Therefore, the ability of calcium ions to form complexes is significant for the stability of the vitamins, and it is likely that the calcium and vitamin D₃ content influences the binding affinity of vitamin D (Jelić et al., 2024). Also, enhanced stability of vitamin D when encapsulated within an amylose inclusion complex was previously reported (Liu et al., 2023). Daly et al.

reported that reduced-fat milk fortified with calcium ions was a very effective carrier for the cholecalciferol, D_3 (Daly et al., 2006). Note that food fortification with vitamin D_3 does not necessarily ensure an adequate supply of the vitamin, as vitamin D is susceptible to chemical/photochemical/thermal degradation and structural changes. Therefore, the choice of vehicle in the fortification process is crucial, as it should provide a protective role for vitamin D and have a form of beadlet (Maurya et al., 2020).

3.1.3.2. Kinetic analysis of calcium carbonate-based vehicles loaded with vitamin D. The most effective approach for evaluating the thermal stability of vitamin D is kinetic analysis, which involves determining the so-called kinetic triplet: activation energy (E_{θ}), pre-exponential factor (A), and reaction mechanism (f(α)). Kinetic analysis can be performed using two main approaches: model-free and model-fitting methods. The model-free approach, also known as the isoconversional method, provides values for E_{θ} and A without assuming a specific reaction model or,

in some cases, by assuming a first-order reaction. Various integral and differential model-free methods are commonly employed for this purpose. (Jelić, 2021). The software Kinetics2015 uses expanded Friedman and assumes the first-order reaction. Non-isothermal kinetics were employed not only in accordance with ICTAC recommendations, as previously stated, but also for practical reasons, given that most reactions in food systems occur under non-isothermal conditions, such as during heating or cooling processes (van Boekel, 2008). Fig. 6a presents the Ea values for all explored vitamin D systems, obtained by expanded Friedman. The Ea is given as a function of conversion degree, α , assuming 1st order. As seen, values of Ea coincide well with thermal stability based on the thermal degradation temperature for vitamin D. Unmodified vehicles loaded with D3 showed lower Ea comparing to modified ones. For unmodified vehicles, Ea is mostly constant in the 0.2 $< \alpha < 0.8$, and for modified ones in 0.2 $< \alpha < 0.6$ range. The value of Ea is considered to be constant for +/-20 % from the average value of Ea (Vyazovkin, 2021). As recommended, the Ea values at $\alpha = 0.1$ and 0.9 are excluded, due to high sensitivity. The E_a values for the systems: (D₃/ CaCO₃)_{COPR}, (D₃/CaCO₃)_{ADS}, (D₃/vit E/CaCO₃)_{COPR} and (D₃/PVA/ $CaCO_3)_{ADS}$ are amounted to 133.5, 130.0, 147.3 and 156.7 kJ mol⁻¹, respectively. In the field of food fortification, Ea plays a critical practical role, as it represents the energy barrier that must be overcome for a reaction to occur, thus without a sufficient activation barrier, undesirable reactions (such as spoilage) would proceed more rapidly, compromising the stability and shelf-life of fortified foods (van Boekel, 2008).

A previously reported activation energy (E_a) value of 131 kJ mol⁻¹ for the thermal decomposition of vitamin D₃ is nearly identical to that observed for D₃ loaded onto unmodified vehicles (Tsai et al., 2017). In contrast, modified vehicles, specifically (D₃/VitE/CaCO₃)_{COPR} and (D₃/ $PVA/CaCO_3)_{ADS}$, exhibited higher E_a values. These results strongly support the findings regarding the enhanced thermal stability of D₃ and align well with the observed decomposition temperature patterns. This suggests that the increased thermal stability of vehicle-loaded vitamin D₃ may be governed by activation energy, implying that a higher energy input is required to disrupt the $D_{\ensuremath{\texttt{3}}}$ molecular structure. Such findings might be very significant for the fortification process, i.e., pasteurization. It was proven that pasteurization can enhance degradation of vitamin D, with losses around 10 to 20 % (Gomes et al., 2016). Ložnjak et al. reported on higher preservation of vitamin D if lower temperatures are used (Ložnjak & Jakobsen, 2018), which goes in favor of vehicle employment.

However, E_{θ} is not the only kinetic parameter that explains the enhanced stability ((Jelić et al., 2019; Vyazovkin, 2021). Actually, it is

insufficient for the interpretation of kinetics itself. Thermal stability of the systems can be governed by an increase in E_a , but also by a decrease in factor A, which is responsible for collisions among molecules. Therefore, the dependence of factor A vs. conversion degree (a) needs to be taken into consideration. Fig. 5b presents the dependence of lnA vs. conversion degree (α) for all the systems under investigation. As seen in this figure, the highest values of lnA were obtained for (D₃/PVA/ $CaCO_3$)_{ADS}, while the lowest one for the $(D_3/vitE/CaCO_3)_{COPR}$. The high values of ln A for $(D_3/PVA/CaCO_3)_{ADS}$ are somehow expected due to the well-known compensation effect, but for (D3/vitE/CaCO3)COPR this could mean that the stability of this system is not governed at all by Ea parameter, but rather by collision factor A. A similar observation was reported by Jelić et al. for the poorly water-soluble drug indomethacin (IND) in the form of an amorphous solid dispersion. Depending on the molar ratio of PVP, the thermal stability of IND was governed by different mechanisms (Jelić et al., 2019).

The best way to estimate individual effects of E_a and lnA on the stability of systems under investigation is through the evaluation of a constant rate. Using Arrhenius equation $k = A \cdot e^{E/RT}$ the constant rates were calculated and given in the following order, from lowest to the highest: k [$(D_3/vitE/CaCO_3)_{COPR}$] < ($D_3/PVA/CaCO_3)_{ADS}$ < ($D_3/CaCO_3)_{COPR}$ < ($D_3/CaCO_3)_{ADS}$. It is clear that the thermal degradation of vitamin D is slower on modified calcium carbonate vehicles.

In order to do kinetic analysis in more detail and to obtain the kinetic triplet in total, evaluation of the reaction model f(a) was conducted with the help of the software feature, Masterplots (Luo et al., 2021). The procedure consists of fitting the master plots from the expanded Friedman method and master plots derived from theoretical models. Best fit is considered to be the optimal kinetic mechanism function. The master plots are usually plotted as a function of activation energy. In Fig. 6, master plots for the systems under investigation are presented. The best fit of experimental data presenting the degradation stage of vitamin D is with diffusion reaction models. Only the system (D₃ CaCO₃)_{ADS} did not show a reliable fitting.

However, there are discrepancies between such an obtained reaction model $f(\alpha)$ and the literature. Zareie et al., by determining the kinetics of vitamin D in fortified canola oil, found that the R_n models, zero and first order kinetics, gave the best fit (Zareie et al., 2021). First-order kinetics are commonly observed in food-related reactions, whereas zero-order kinetics typically characterize quality changes such as brown color development, often associated with processes like the Maillard reaction (Kathuria et al., 2023; van Boekel, 2008). Rakusa et al. investigated, in a systematic fashion, the influence of temperature, pH, light, oxygen, etc.,



Fig. 5. a (left) and Fig. 6b (right). Activation energy and preexponential factor A for the systems: $(D_3/CaCO_3)_{COPR}$ – black square, $(D_3/CaCO_3)_{ADS}$ – blue triangle, $(D_3/vit E/CaCO_3)_{COPR}$ – red circles, $(D_3/PVA/CaCO_3)_{ADS}$ – green upside down triangle.



Fig. 6. Master plots for $(D_3/CaCO_3)_{COPR}$ – down right, $(D_3/CaCO_3)_{ADS}$ - down left, $(D_3/vit E/CaCO_3)_{COPR}$ – upper right and $(D_3/PVA/CaCO_3)_{ADS}$. – upper left; Diffusion model (blue line), N-th order model (red line) and Nucleation and growth model (green line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

on vitamin D stability in aqueous media using the HPLC-UV method. She found that first-order kinetics gave the most satisfactory results except in the case of light and oxygen influence, for which the Weibull distribution energy model was chosen (Rakuša et al., 2021). Tsai et al. also reported *n*-th model as the most suitable for thermal decomposition of vitamin D with the following kinetic parameters: $E_a = 131 \text{ kJ mol}^{-1}$, n = 1.22, $\ln A = 27.98$ and $\Delta H = 134.4$ (Tsai et al., 2017). Even though master plots are very convenient for the determination of reaction mechanism, there are some restrictions, such as the activation energy must not vary in the α range.

Therefore, we conducted the model fitting approach, and the results are listed in Table 1. As seen, the best model to describe the degradation

 Table 1

 Kinetic parameters for unmode

Kinetic parameters for unmodified and modified calcium carbonate vehicles loaded with vitamin D_3 using model-fitting approach (N-th order model).

N-th order model	$E_{\rm a}$, kJ mol ⁻¹	A, s^{-1}	n
Adsorption D ₃ /CaCO ₃	107.7	$2.9 \cdot 10^{8}$	2.12
Adsorption D ₃ /PVA/CaCO ₃	196.4	$4.8 \cdot 10^{16}$	1.83
Coprecipitation D ₃ /CaCO ₃	127.5	$2.0 \cdot 10^{10}$	1.16
Coprecipitation D ₃ /vit. E/CaCO ₃	89.7	$1.1 \cdot 10^{6}$	0.66

process of vitamin D proved to be N-th order model, which agrees well with the literature data. It is interesting to note, that results from model fitting method confirmed our findings from model free approach, i.e. thermal stability of D₃/PVA/CaCO₃ is governed by activation energy (196.4 kJ mol⁻¹), and for stability of D₃/vit E/CaCO₃, factor A (1.1·10⁶ s⁻¹) seems to be responsible.

Since we have calculated kinetic parameters and found the most appropriate kinetic model by fitting approach, we can estimate the shelf-life of vitamin D loaded on unmodified and modified calcium carbonate vehicles (Calvino et al., 2021; Jelić et al., 2021). Similar procedure was applied for paracetamol's and ambroxol hydrochloride's shelf life. Specifically, t_{α} can be can be calculated by the following equation:

$$t_{\alpha} = \frac{g(\alpha)}{A \exp\left(-\frac{E}{RT_0}\right)} \tag{4}$$

where T_0 is the fixed temperature (25, 40, and 70 °C), while $g(\alpha)$ is a function dependent on the specific reaction mechanism, in our case, N-th order model. Note that shelf life here is evaluated only in terms of thermal degradation in a neutral, N₂ atmosphere. Other interfering factors, such as air, humidity, and oxygen, have not been taken into

consideration. However, it is evident that the shelf life of vitamin D is dependent on the encapsulation process and the structural properties of the selected carrier. From a practical perspective, this approach (also known as accelerated) offers a significant reduction in both the time and cost typically associated with conventional shelf-life testing (Jelić, 2021; Modhave et al., 2024; Ntzimani et al., 2025). Fig. 7 presents the shelf-life bars based on eq. 3 at the following temperatures: 25, 40, and 75 °C.

The Ca^{2+} ion, with a coordination number of 8, demonstrates a strong binding affinity toward water and specific organic ligands. Calcium's pronounced capability to form complexes with thermally unstable compounds has been substantiated and may hold significance in the fortification process. Vitamin D interacts with metal ions via its hydroxyl group, and extensive research has been conducted on the formation of metal-vitamin D complexes. Abbasi et al. reported enhanced stability of vitamin D when encapsulated in whey protein due to complexation (Abbasi et al., 2014), while Loewen et al. demonstrated that the incorporation of vitamin D into casein micelles is dependent on calcium content (Loewen et al., 2018). From a stability perspective, hydrophobic interactions (ligand-ligand interactions) that include long hydrophobic segments that could bind to the hydrophobic rings of steroids through van der Waals forces must be taken into consideration. In this way, the thermal stability of vitamin D can be additionally enhanced. Such interactions may be feasible in (D3/PVA/CaCO3)ADS and (D₃/VitE/CaCO₃)_{COPR}, potentially arising from interactions between two vitamin D molecules or between vitamin D and vitamin E, respectively. The ability of calcium to enhance thermal stability, extend shelf life, and improve the loading capacity of vitamin D could offer substantial benefits for fortification. Moreover, kinetic analysis has proven to be a highly effective tool for quantifying these calcium-mediated effects.

4. Conclusion

The present study emphasizes two primary contributions: (1) the design of a simple, efficient, scalable, and cost-effective vehicle for vitamin D_3 fortification; and (2) the successful utilization of kinetic computational modeling.

The novelty of this research lies in the development of newly engineered nanoscale solid particle vehicle systems that possess distinct physicochemical characteristics and surface morphologies, resulting in a high adsorption capacity for the model compound, vitamin D₃. The two modified delivery vehicles, namely $(D_3/PVA/CaCO_3)_{ADS}$ and $(D_3/VitE/$ $CaCO_3)_{COPR}$, were synthesized with the objective of enhancing the stability of vitamin D₃. As such, these systems demonstrate potential as functional ingredients for application in food supplementation and fortified food products. Given that most foods exist in nonequilibrium states, a comprehensive understanding of both thermodynamics and kinetics is essential to determine the feasibility of transformations within the food matrix, as well as to evaluate their rate and extent. Information on the stability, safety, quality, and shelf life of vitamin D_3 -enriched food systems was derived through kinetic computational modeling.

The thermal stability of $(D_3/PVA/CaCO_3)_{ADS}$ was primarily influenced by the activation energy, whereas for $(D_3/VitE/CaCO_3)_{COPR}$, the pre-exponential factor (A) played a dominant role, suggesting distinct underlying reaction mechanisms. Among the tested kinetic models, the N-th order model provided the best fit to the experimental data. The shelf life of vitamin D₃ was found to be significantly affected by both the encapsulation method and the structural characteristics of the chosen delivery vehicle. Furthermore, the ability of calcium ions to participate in complexation processes facilitated the incorporation of multiple vitamin D₃ molecules into the calcium carbonate-based vehicle and promoted interactions between the two hydrophobic compounds, vitamin D₄ and vitamin E.

Future research should focus on evaluating the behavior and performance of these delivery systems in complex, real food matrices under actual industrial processing and storage conditions, including factors such as oxygen exposure, moisture content, temperature fluctuations, and light-induced degradation. Addressing these variables is essential for validating the practical applicability of the proposed systems beyond the model environment used in this study. In addition, the integration of advanced computational tools, such as machine learning and artificial intelligence, could further improve the predictive power of kinetic modeling, allowing for the optimization of formulation parameters and processing conditions in a more efficient and cost-effective manner.

The results of this study provide a solid scientific foundation for the application of engineered nanoscale solid-particle systems in food fortification. These systems offer the potential to improve vitamin D_3 stability, enhance the functional performance of fortified products, and extend shelf-life. Furthermore, by leveraging kinetic modeling and material design, these strategies can contribute to reducing production costs, supporting regulatory compliance, and fostering innovation in the development of next-generation fortified foods that meet both industry and consumer needs.

CRediT authorship contribution statement

Dijana Jelić: Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Conceptualization. Milan Vraneš: Writing – review & editing, Validation, Software, Investigation, Formal analysis, Data curation. Saša Zeljković: Writing –



Fig. 7. Shelf-life bars of vitamin D in investigated systems at following temperatures: 25, 40 and 75 °C.

review & editing, Validation, Investigation, Formal analysis, Data curation. **Suzana Veličković:** Writing – review & editing, Validation, Investigation, Formal analysis, Data curation. **Filip Veljković:** Writing – review & editing, Validation, Investigation, Formal analysis, Data curation. **Aleksandar Cvetkovski:** Writing – review & editing, Validation, Investigation, Formal analysis, Data curation. **Biljana Pejova:** Writing – review & editing, Validation, Investigation, Formal analysis, Data curation. **Snežana Papović:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

D.J. and S.Z. are thankful to Ministry of Scientific and Technological Development and Higher Education of Republic of Srpska for supporting this study through project No.19.032/961-96/23. D.J. is grateful to Academy of Sciences and Arts of the Republic of Srpska and Serbian Academy of Sciences and Arts for supporting the study through interacademic cooperation project"Kinetics of reaction on phase boundary gas/solid - thermal stability of metal oxides and pharmaceutical products in oxidation and reduction atmosphere". D.J. gratefully acknowledges the University of Banja Luka and Rector prof. dr. Radoslav Gajanin for their support of postdoctoral research in Japan. M.V. and S.P. would like to thank Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-137/2025-03/ 200125 and 451-03-136/2025-03/200125) and Secretariat for Higher Education and Scientific research of the Autonomous Province of Vojvodina (Grant No. 142-451-2545/2021-01/2). Special thanks to Sanja Pržulj and Teodora Knežević for technical experimental support and prof. dr Srđan Rakić from the University of Novi Sad, Faculty of Sciences for XRD analysis.

Data availability

No data was used for the research described in the article.

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