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Study of UV and thermal stability of Vitamin D3 loaded in yeast cells of *Saccharomyces cerevisiae* microcapsules

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Abstract

Background and objective: Vitamin D is a fat soluble nutrient which was help to improve human health. There are different problems in VD_3 fortification of foods including low water solubility and susceptibility to environmental conditions. Microencapsulation of VD_3 could be used to overcome these limitations. Encapsulation of VD_3 in yeast cells can be counted as an affective technique to provide a protection against VD_3-photochemical degradation.

Material and methods: In this study, VD_3 was encapsulated in *Saccharomyces cerevisiae* yeast cells as encapsulating matrix. Microcapsules were dried by spray drier and freeze drier. Two concentration of vitamin (100 and 500 IU) used for bread fortification. Furthermore, the effect of UV irradiation and thermal treatment (80 °C for 1 minutes) on stability of free and encapsulated vitamin D was investigated.

Results and conclusion: The results showed, about 40% and 95% of vitamin were remained after application of UV and high temperature treatment, respectively. In addition, yeast cells could provide good protection against (more than 95%) high temperature of bread baking process (180 °C for 30min). The analysis of bread quality and sensory properties showed higher value of bread containing encapsulated VD_3 in comparison to free form. The results of this study show that the *Saccharomyces cerevisiae* yeast cell could be used as a suitable carrier for encapsulation of VD_3.

Keywords: vitamin D, bread, yeast cells, *Saccharomyces cerevisiae*

1. Introduction

Vitamin D (VD3) deficiency is a concerning

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problem in world which has spread in both poor and industrialized countries. According to previous data published, low VD3 status is common in all age groups [1]. In addition, usually people who do not get enough exposure to sunlight, and also people who suffer from digestive diseases, suffer from vitamin D deficiency [2]. It was reported that nearly 40% of adult population have low and insufficient status of VD3 all over the world [3]. This public health problem can cause Osteoporosis and Osteomalacia in adults and also cause Rickets in children [4]. Cholecalciferol (vitamin D_3) also has important effects on calcium and phosphor absorption and the health of bones and teeth [5, 6]. It contributes to transform of calcium and phosphor to the bones and reabsorption of these minerals in kidney [7]. Many recent studies pointed out, VD3 has protective effects against many different diseases such as diabetes, hypertension, cardiovascular diseases, multiple sclerosis (MS), cancer and bacterial infections [2, 8, 9].

There are limited natural sources of VD3 in foods and in small amounts of it (egg yolk, fish oil and beef liver, mycoprotein) [4, 10, 11]. Therefore, fortification of foods with VD3 get more attention recently. The food that was selected as carrier of fortified nutrients should have widespread people consumption [12]. So, the bread was chose to fortify by cholecalciferol due to high consumption and more accessibility in different societies. VD3 is sensitive to food processing including harsh temperature and other environmental stresses such as oxygen. Additionally, it cannot be added to aqueous foods unless we encapsulate it into a proper and water soluble carrier. Yeast cells of *Saccharomyces cerevisiae (S. cerevisiae)* are possible carriers to overcome these problems. The cell wall of *S. cerevisiae* consist of a network of 1, 3-β glucans and 1,6-β glucans and mannoproteins [13, 14]. It has been declared that it has preservative effect on the encapsulated substance [15, 16].

This study is an investigation on stability of vitamin D_3 loaded yeast cells against temperature and UV exposure. Also we assessed the sensory properties of fortified bread with these microcapsules and measured the VD3 stability after bread baking.

2. Materials and methods

2.1. Materials

Pure cholecalciferol (> 99%) was purchased from DSM (DSM, Switzerland). All the chemicals used for HPLC analysis were HPLC grade and purchased from Merck Co (Darmstadt, Germany). The commercially yeast cells (Fariman, Mashhad, Iran) were used as carrier in microencapsulation and as fermentation agent in bread making. Flour (Jonob Flour Co, Iran), high purity sodium chloride (Spidan Co, Iran), bread improver (Omaj Co., Iran) were used for bread manufacturing. Potassium hexaferrocyanide and zinc acetate were purchased from Panreac (Belgium). To prepare Carrez solution I (0.25 mol L-1), 10.6 g of potassium hexacyanoferrate was dissolved in 100 mL distilled water. Carrez solution II was prepared at the concentration of 0.4 mol L-1 using added 21.9 g of zinc acetate to 3 mL of acetic acid, then adjusting its volume to 100 mL with distilled water. The pH of the solutions was adjusted using a diluted hydrochloric acid solution. All other chemicals used were of analytical grade or were of the highest purity.

2.2. Preparation of yeast microcapsules containing vitamin D3

We prepare microcapsules using two different drying methods and also some of the treatments were prepared by plasmolysed yeast cells. For initial preparation of yeast cells, dry yeast cells after rinsing with phosphate buffer solution and then deionzed water, were lyophilized with a freeze drier (ALPHA 2-4; Christ, Harz, Germany) or in a spray dryer (BÜCHI, Mini Spray Dryer B-290, Germany). To prepare the plasmolysed yeast cells, washed cells were suspended to 10% NaCl solution and let it shake for 48 h at 55 ºC and 180 rpm. This step followed by

washing cells by water to remove released materials and remained NaCl.

Microencapsulation process is as follows: in order to prepare each treatment, 10 mg of dried cells (plasmolysed or non-plasmolysed) were added to 70 ml deionized water and VD3 solution to reach a weight ratio of 2.5 mg and 12.5 mg VD3 per gram of yeast cells (100000 and 500000 IU/g yeast, respectively). The suspension was incubated at 40 ºC for 12 h and 180 rpm shaking. After harvesting microcapsules by centrifuge (6000 rpm, 10 min), microcapsules were washed by deionized water and dried by freeze drier (frozen at -20 ºC and then lyophilized for 14 h) or spray drier (inlet temperature 130 °C, outlet temperature 75-77 °C, feed flow rate 6.08 mL min^{-1}, nozzle diameter 0.7 mm, dry air flow rate 568 L h^{-1}, aspirator 90% and pump rate 25%) [17]. Treatments codes are as follows: S-100 (microcapsule containing 100000 IU VD3 and spray dried), S-500 (microcapsule containing 100000 IU VD3 and spray dried), F-100 (microcapsule containing 100000 IU VD3 and freeze dried), and F-500 (microcapsule containing 100000 IU VD3 and freeze dried).

2.3. Fortified bread samples preparation

Wheat flour with 72% extraction rate was provided from local supermarkets (Tehran, Iran). To prepare each sample, a mixture of 1.5 g dry yeast, 1 g sodium chloride, 2 g sugar and 1.5 g bread improver were added to 150 g of flour. Then 60 ml deionized water was added to the mixture. VD3 (1000 IU/ 50g) was added to dough in form of encapsulated (S-100, S-500, F-100 and F-500) or free. After making a good mixture, let the dough rise for 1h in 35 C˚. Then sample were put in baking dish and baked in 180 C˚ for 20 minutes.

2.4. High performance liquid chromatography (HPLC)

The quantification of cholecalciferol performed by HPLC (Agilent technologies, UK) with CE-4200 UV-Vis detector (Cambridge, UK) and six-port valve (Rheodyne, USA). It has equipped with two CE-4100 pumps, multiple solvent delivery unit, vacuum degasser, mixing chamber. Methanol used as mobile phase at a flow rate of 1.0 ml/min and the injection volume was 20 µl. An ODS Column (250 mm× 4.6 inner diameter, 5 µm particle size) from Phenomenex (Torrance, CA, USA) was used. The column temperature was 25 ºC and the quantification was performed at 265nm. Concentration of cholecalciferol was assessed by standard curve, obtained from standard solution of VD3. The total run time was 28 min.

2.5. Extraction of vitamin and quantification

VD3 content of breads was determined following the procedure outlined by kamankesh et al., (2016) with slight modifications. Two g of powdered bread sample was weighted and put into a 50 ml falcon, this sample spiked with VD3 in 10 ml of KOH (1 mol L-1)-ethanol solution containing 2% w/v sodium ascorbate was added and let it shake to hydrolyze the sample. After that, for better extraction of VD3 and transferring it to aqueous phase, the container of sample was immersed into an ultrasonic water bath at 40 kHz of ultrasound frequency and 0.138 kW of power for 6 min at 25C˚. Sample solution was centrifuged (5000 rpm/5 min), supernatant was separated and transferred for pH adjustment to 4.5. After adjusting pH, 1 mL potassium hexaferrocyanide (Carrez solutionI) and 1 mL zinc acetate (Carrez solution II) were added to precipitate the protein. The mixture was shaken for a few minutes and then centrifuged (5000 rpm/5 min). The obtained supernatant was immediately used to the DLLME process. Under optimum conditions, 650 µL of disperser solvent (ethanol) and 100 µL extraction solvent (1-octanol) were directly injected into a test tube containing 10 mL of sample solution. The mixture was shaken for 1 min using shaker and then centrifuged (5000 rpm/5 min). After this process, 1-octanol floated on the aqueous sample. Approximately 60 µL of 1-octanol was separated using a syringe, and 20 µL of it was injected into the HPLC using a microsyringe.

2.6. UV stability of encapsulated vitamin D

Yeast microcapsules loaded vitamin D3 were placed in transparent glass vials and exposed to UV light in a laminar hood cabinet for 3.5 h at room temperature. 20 mg from each sample were collected, periodically (0 h, 0.5 h, 1.5 h, 2.5 h, 3.5 h). 8ml methanol and 2 ml deionized water were added to samples for vitamin D extraction. Let it vortex overnight and then centrifuged (10 min / 5000 rpm). Supernatant were filtered using a 0.45 µm PVD3F filter followed by quantification with HPLC.

2.7. Thermal stability of vitamin D loaded yeast cells

Each sample of microcapsules diluted to deionized water to yield final VD3 concentration of 10 µg/ml. 1 ml of each suspensions were accurately weighted in glass tube protected from light. Also there was a tube containing unprotected VD3 in deionized water, at the same final VD3 concentration. Samples were prepared in triplicate to eliminate errors. All 15 tubes were put in 80C° bath water for 1 minute and then immediately chilled in ice-water. VD3 content was determined before and after heating using solvent extraction and HPLC analysis (section 2.6).

2.8. Differential scanning calorimetry (DSC)

The DSC thermograms of the samples were obtained by a differential scanning calorimetry instrument (Shimadzu Co., Japan). For this purpose, 6-12 mg of the samples were attached on aluminum pans and heated at temperature range from 20–300 °C under nitrogen atmosphere with flow rate of 30 ml min^{-1} and at scanning rate of 10 °C min^{-1}.

2.9. Sensory properties

Sensory properties were determined using the AACC (2000) method (10-90) by a verbal hedonic scale including five points (1: Disliked extremely; 5: Liked extremely). Taste and odor, color, texture and total acceptance of bread samples were assessed by 25 consumers. Final score was obtained by next equation:

$$Final\ score = Total\ experience/Total\ coefficients$$

Treatments containing free and encapsulated VD3 were compared with control sample that has no vitamin.

2.10. Statistical analysis

All measurements were performed in triplicate. The statistical processing of the obtained data was performed by SPSS, version 21 (IBM Corp., Armonk, NY, USA). Data were expressed as means ± standard deviations. To determine any significance differences between treatments, one-way analysis of variance (ANOVA) followed by the Duncan's multiple range procedure was used at a level of 95% of confidence (α =0.05).

3. Results and discussion

3.1. UV stability of encapsulated vitamin D

As demonstrated in Fig. 1 Encapsulated VD3 showed a good stability to UV exposure in compare to control sample. The remained amount of unprotected VD3 after 3.5 h was 22.9% due to photochemical degradation of vitamin. Whereas more than 53% of VD3 were intact in encapsulated samples. Encapsulation of VD3 in yeast cells can be counted as an affective technique to provide a protection against VD3-photochemical degradation. It can be due to UV absorbance of proteins and carbohydrate of yeast cell wall which reduced intensity of rays that reach to VD3. There is similar study which said that when vitamin D3 encapsulated in zein nanoparticles, the rate of photochemical degradation decreased and the remained vitamin after exposing to UV-light increased in compare to free vitamin [18]. Also there is a report about vitamin D2 in which vitamin was encapsulated in soluble soy protein isolated and it increased UV-stability of VD2 [19]. Semo et al. (2007) reported casein micelles can protect VD2 and retard its degradation in presence of UV light [7].

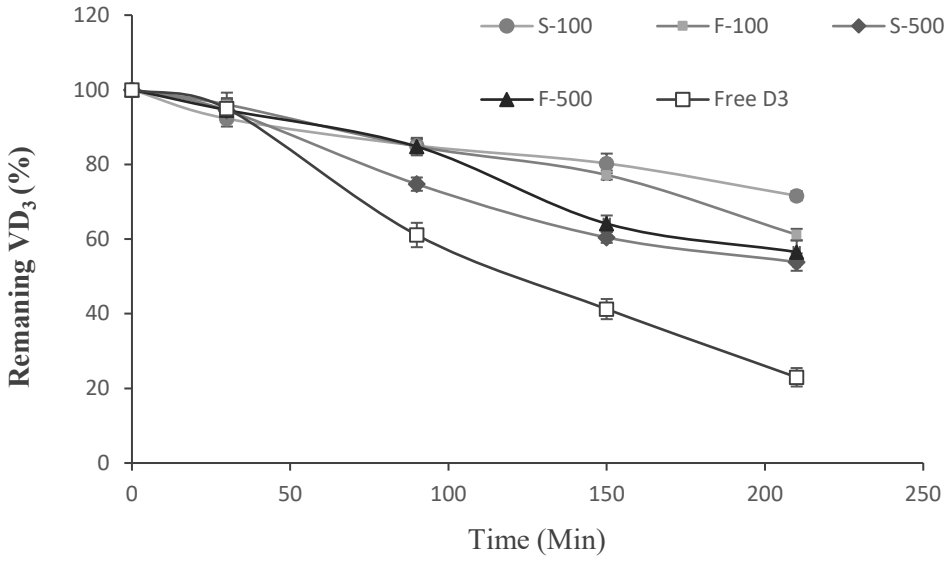

Fig. 1: Photochemical stability of VD3 against UV light for 210 min

3.2. Thermal stability of vitamin D loaded yeast cells

Doing a thermal operation at 80 ºC for 1 min revealed data which is depicted in Fig. 2 Free VD3 sample showed significant thermal degradation. All four encapsulated samples were almost stable to this thermal treatment. The remained vitamin concentration was more than 90% for encapsulated VD3 in yeast cells. While 62% of unprotected vitamin was lost during the thermal operation. It was mentioned that yeast cells can protect the inside material up to approximately 265 ºC [20]. Our data are in accordance with data published by Yonatan Levinson et al. (2014) mentioned that the thermal stability of vitamin D increased by vitamin encapsulation in soybean protein [21].

3.3. DSC studies

Thermal behavior of samples was investigated by DSC analysis. DSC is a useful technique to monitor the effect of different additives on thermal properties of materials, and used to provide qualitative information about the physicochemical status of the core material in the various matrixes [22]. Fig. 3 displays DSC curves of vitamin D_3, unloaded and loaded yeast cells. The DSC thermogram of VD3 showed an endothermic peak at 87.8 °C corresponding to its melting point. Similar observations have been reported [18]. Compared to non-plasmolyzed and plasmolyzed yeast microcapsules, the endothermic peak in plasmolysed yeast cells shifted to lower temperature. This decrease in phase transition temperature might be because of the increasing of the fluidity of yeast cell wall due to plasmolysis [23]. Thermal behavior of microcapsules was not influenced by the VD_3 incorporation. In addition, no new peak was detected in VD3-loaded microcapsules, which may be due to the absence of vitamin in residual crystalline form.

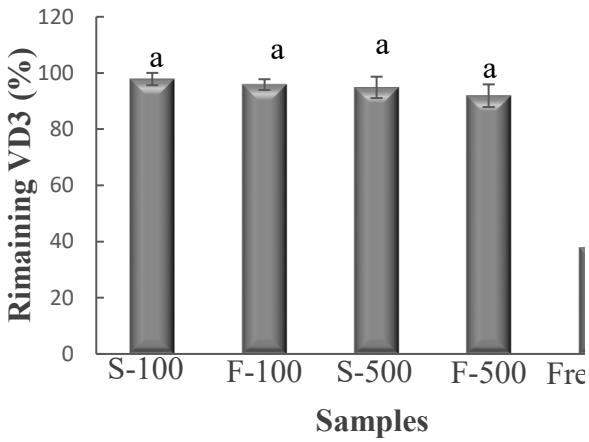

Fig. 2: Percentage of remaining VD3 after Heat treatment at 80 °C for 1 min. Significant differences between treatments are indicated by different letters on each column ($P < 0.05$).

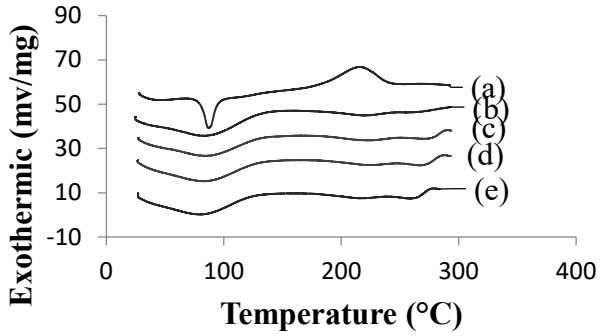

Fig. 3: DSC spectra of VD3 (a), unloaded non-plasmolysed cell microcapsules (b), unloaded plasmolysed cell microcapsules (c), VD3-loaded non-plasmolysed cell microcapsules (d), and VD3-loaded plasmolysed cell microcapsules (e).

3.4. Stability of vitamin D in bread after baking

Assessment of the quantitation of VD3 in bread samples after baking resulted data showed in Fig. 4 Using encapsulated VD3 had good protective effect against baking temperature.

About 98%, 97%, 96% and 94% of VD3 were detected in baked bread in samples S-100, F-100, S-500, F-500 respectively. While 34% of free VD3 were remained in bread after baking dough. Most of VD3 were decomposed in high backing temperature. Tabibian et al. reported that VD3 in fortified flat bread was decreased by increasing baking temperature [24].

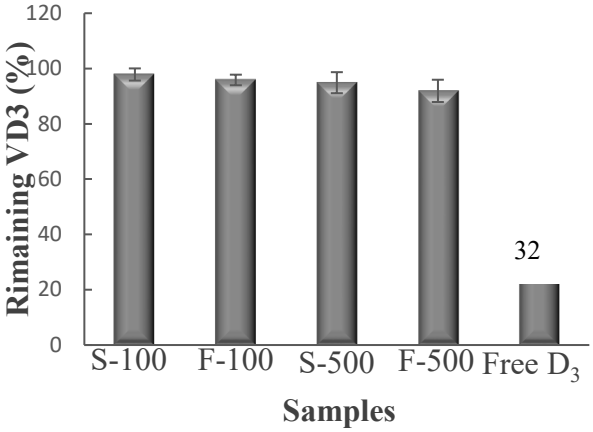

Fig. 4: The percentage of VD3 remaining in different bread samples after the baking process.

3.5. Sensory properties

Sensory evaluation of bread samples was done by 25 persons. In taste, odor and color properties, both samples containing encapsulated VD3 showed no significant difference with control. In compare, free VD3 containing bread get low accepting (Table 1). As adding VD3 made no changes in texture of bread, the texture of all samples showed no significant difference. In total acceptance, free VD3 containing bread get low accepting from other samples. While control and encapsulated samples had no difference in total acceptance.

Table 1 Sensory evaluation of the bread without VD3 (control) and with free and encapsulated VD3 (S-100 and F-100).[a,b]

Bread	Sensory quality score			
	Taste and odor	Texture	Color	Overall acceptance
Control	4.25±0.55[a]	4.05±0.68[a]	4.30±0.73[a]	4.30±0.47[a]
Free VD_3	3.15±0.48[b]	3.75±0.63[a]	3.00±0.45[b]	3.15±0.58[b]
S-100	4.15±0.74[a]	4.10±0.71[a]	3.95±0.75[a]	4.00±0.64[a]
F-100	4.10±0.66[a]	4.06±0.85[a]	4.15±0.77[a]	4.15±0.54[a]

[a] Values within each column with different letters are significantly different ($P < 0.05$).

[b] Data reported are average values ± standard deviations.

4. Conclusion

Yeast cells loaded by vitamin D3 were prepared and dried capsules by spray and freeze drier. Bread samples were made and fortified with capsules in two concentration of vitamin (100IU/500IU) and free vitamin D3. Presence of vitamin in microcapsules were proved by DSC analysis. Assessment of thermal stability of vitamin in bread samples showed that 90% of vitamin was protected after applying 80 ºC for 1 minute. Also capsules showed well protection against UV exposure. More than 53% of protected vitamin was remain in bread in compare to 22.9% for control sample. Baking temperature (180 ºC/30 min) caused about 6% vitamin loss of encapsulated vitamin in dough while it is 66% for free vitamin. This is due to vitamin degradation in high temperature. 25 persons tested fortified and non-fortified bread samples. Results demonstrated that fortification had no effect on texture of bread but in taste odor and color properties bread samples containing microcapsules get higher acceptance that those with free vitamin D3.

4. Acknowledgment

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5. Conflict of interest

The authors declare that there is no conflict of interest.

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