Iranian Food Science and Technology Research Journal
Vol. 16, No. 3, Aug. Sep. 2020, p. 37-50

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد 16، شماره 3، مرداد - شهریور 1399، ص. 50-37

Research Full Papers

Vitamin protection by Alginate-Whey Protein Micro Gel (AL-WPC MG) as a novel microcapsule against gastrointestinal condition; case study: B-complex vitamins.

M. Zandi*

Received: 2019.02.23
Accepted: 2019.09.01

Abstract

The aim of the current research was to identify and develop an ideal delivery system in order to protect the vitamin from gastrointestinal conditions. For this purpose, vitamin loaded Alginate-Whey protein micro gels (AL-WPC MGs) developed as a biopolymer carrier. This microcapsule was examined in terms of morphology, ζ-potential particle size and distribution, encapsulation and delivery efficiency, and in vitro gastric and intestinal digestions. Absorbance method was used to monitor B-complex vitamins release over time at the simulated gastrointestinal conditions. Release experiments illustrated beneficial attributes for these microspheres. Release mechanism was predicted by using various kinetic equations. Results indicated that the most of the fabricated spherical shaped AL-WPC MGs was under 100 μm in size, and these microcapsules had an excellent and moderate stability in gastric and intestinal conditions, respectively. It was found that the highest vitamin release rate occurs in the simulated gastric-intestinal situation, and type of the vitamin had a slight effect on the release rate and release profile. Kinetic models suggested that release from group B vitamins mainly was controlled by Fickian diffusion mechanism. In general, this research showed that the AL-WPC MGs protect the vitamin from gastric digestion and could be used as a delivery system.

In previous works, a novel AL-WP MGs and use for different active agent encapsulation was developed, while the final purpose of this work was to study the vitamin release mechanism from AL-WPC MGs at the gastro–intestinal situation. Accordingly, this microcapsule showed the highest vitamin release rate at the simulated intestinal situation. This high release could be due to instability of alginate in neutral pH, and also enzymatic digestion of whey protein. The better release of vitamin at intestinal condition is desirable to achieve the nutrient effect during food consumption. This micro gel therefore appears to be potentially beneficial as digestion delivery vehicles for bioactive compounds in the food and nutraceuticals industry as well as non-food industry.

Keywords: B-complex vitamin, control release, micro gel, whey protein, alginate

Introduction

Vitamins as a micronutrient are a group of organic compounds that are needed in small quantities for the body metabolism to work properly and stay healthy. Vitamins are classified into two categories including fat soluble (vitamins A, D, E, and K) and water soluble (vitamins C and the B-complex vitamins). Water-soluble vitamins are a sensitive and cannot stay in body. One of the water-soluble vitamins are the group B (or B complex) vitamins, which has vital roles in metabolic processes such as a red blood cell formation and energy production. B-complex vitamins classified into 8 categories including B_1 (thiamine), B_2 (riboflavin), B_3 (niacin), B_5 (pantothenic acid), B_6 (pyridoxine), B_7 or H (biotin), B_9 or B_{11} of M (folate), B_{12} (cobalamin) (LeBlanc et al., 2011; Beck, 2001; Molina et al., 2009). Many cereals are one of the richest sources of B complex vitamins; however fish, poultry, meat, eggs, dairy products, Leafy green vegetables, beans, peas also has a good level of group B vitamins (Moll and Davis, 2017; Beck, 2001).

* Department Food Science and Engineering, Faculty of Agriculture, University of Zanjan, Zanjan, Iran.

(-Corresponding Author: Zandi@znu.ac.ir)
DOI: 10.22067/ifstrj.v16i3.79215

Many of these nutrients are essential to regulate vital biochemical reactions in the cell and cannot synthesize in the living organisms or synthesize in insufficient level; therefore, most of them should be provided by diet. In the recent decade, vitamin deficiencies occur in many societies because of malnutrition or unbalanced diets; thus, fortification of food with vitamins is necessity. Although most of the natural food substance (unprocessed food) has enough vitamin level; but usually food processing and storage cause the greatest vitamin loss. When, food passing from gastrointestinal system, nutrient exposed to the hard condition such as an acidic pH and easily destroyed. Due to the decreasing of vitamin loss, there is a need for encapsulation of these micronutrients to protect them from processing, storage and gastrointestinal conditions and also any undesirable interaction or reaction with the environment. This capsule must intelligently act to achieve a lower gastric release but a faster intestinal release (LeBlanc *et al*., 2011; Beck, 2001; Moschona and Liakopoulou-Kyriakides, 2018; Abbasi *et al.,* 2018).

Encapsulation is the best delivery vehicle that enables enhanced the stability and bioavailability of an active agent against the gastrointestinal conditions. Such entrapping vesicular system could release their core material from semi porous shell under the specific situation (namely controlled release). Various shell materials and different methods are being used by researchers for fabrication of special delivery systems that typically have to be particularly designed for each application; however, some of them are effective, safe, cheap, and applicable (Cheong *et al.,* 2016; Fani *et al.,* 2017; Jafari, 2017; Zandi *et al*., 2014; Zandi and Mohebbei, 2014; Zandi *et al.*, 2017; McClements, 2015; Oehlke *et al.*, 2014; Zhang *et al.* 2016). Recently, food grade protein- polysaccharide interaction as a promising delivery vehicle has been considerable interest. Lately, Alginate-Whey protein micro gels (AL-WP MGs) used as biopolymer carriers and candidate for targeted release system. AL-WP MGs are soft and small particle that usually less than 100 μm in size (Lamas *et al*., 2001).

AL-WP MGs were made via whey protein isolated (or whey protein concentration) and sodium alginate using emulsification/ internal gelatin method. Whey protein is extensively used as food ingredients because they have unique properties include high nutritional values, water-binding, foaming stabilizing, emulsion stabilizing, good gel producing, and thickening properties. Whey protein may be used as carriers for hydrophobic substances in food products and pharmaceutical (Leon *et al.*, 2018) (Abbasi *et al.,* 2018). The alginate polymer consists of linear copolymers of β-(1-4) linked D-mannuronic acid and α-(1-4)-linked L-guluronic acid (G) residues which is able to form pH-sensitive and temperature-independent hydrogels. This attractive polymer could be used as a component of a delivery matrix for lipophilic active and bio-active agents (Ni *et al.,* 2015). Ionic crosslinking with cations (ionic gels) or acid precipitation (acidic gels) are used as two methods for alginate gel formation (Ching *et al.,* 2017; Koutina *et al.,* 2018; Bouyer *et al.,* 2012). For active agent encapsulation, sodium alginate solution containing the bioactive is injected into whey protein solution that results in the formation of soft and moist cold AL-WP MGs (Zhang *et al.*, 2016). Due to AL-WP MGs mechanical and viscoelastic properties; these types of microcapsules could use for the nutrient (Zandi, 2017; Zandi *et al.,* 2017) flavors (Zandi *et al.*, 2014), Drug and other active and bioactive agents (Zandi *et al.,* 2017; Abbasi *et al.,* 2018; Chen and Subirade, 2006) encapsulation in a wide range of research and industry applications (Zhang *et al.,* 2015; Zhang *et al.*, 2016). AL-WP MGs can protect the vitamin from the acidic situation in the stomach and make them available in the intestines for increased bioavailability (Wichchukit *et al.*, 2013).

In prior works (Zandi, 2017; Zandi *et al.*, 2014; Zandi *et al.*, 2017; Zandi and Mohebbei, 2015) we developed novel AL-WP MGs and use for different active agent encapsulation. Such microspheres should be degraded by

intestines condition, allowing vitamin release. The model vitamin were group B vitamin. For this purpose, release mechanism, kinetic and profile of an encapsulated any vitamins through the AL-WP MGs shell at the gastrointestinal situation was investigated by spectrophotometry technique; then kinetic models were fitted to the experimental release data for release kinetic prediction. Finally, the influence of consumption condition on the encapsulation, retention and release of the vitamins were then measured.

Method and material

Whey protein concentrate with 80% protein and 4% moisture content was purchased from Davisco Foods International Inc. (USA). Sodium alginate (sodium salt, 99.5%), sodium hydroxide, potassium dihydrogen phosphate, hydrochloric acid, sodium bicarbonate, analytical grade pepsin and pancreatic enzymes, calcium chloride (Sigma Aldrich, St. Louis, MO; > 93%) and deionized water of resistivity 18.2 MΩ·cm were purchased from the Sigma Chemical Company (St. Louis, MO, USA). Tween 80 (Fluka, Switzerland), sunflower oil (from the supermarket), sodium chloride (Fluka) were used without further purification. Thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, folate, biotin and cobalamin were supplied by Sigma Chemical Co., (St. Louis, MO, USA). Double distilled water was used to make all solutions. All other reagents were of analytical grade and acquired from Merck co (Germany).

AL-WP MGs fabrication via Ultrasonication

AL-WP MGs loaded with group B vitamin was prepared based on Zandi et al. (2014, 2017) technique with slighted modification. 2% (w/v) Alginate (AL) solution and 8% (w/v) Whey Protein concentrate (WP) was prepared by dissolving both ingredients separately in deionized water at room temperature under mild agitation for 1 h using a magnetic stirrer (IKAWerke GmbH&Co. KG, model RH basis). The resulting solution was held overnight at 4℃ to ensure complete and proper hydration of the components. WP solution adjusted to pH= 8 and was left at room temperature for 2 h, then it was heated at a temperature controlled water bath at 80°C for 30 min to denature and aggregate the WP. Heating stage facilitates the formation of stable WP emulsion structures. WP solution was cooled and kept at room temperature for 2 h. then WP (80% wt) and AL (20% wt) were mixed and stirred for 30h at room temperature. The obtained formulation was allowed to stand overnight at 4℃ . To prepare VitB- AL–WP emulsion, AL–WP solution (20% v/v), Tween 80 (0.05% v/v)), group B vitamin (0.05% v/v) and sunflower oil (20% v/v) were blended and stirred with a high-speed blender (Ultra Turrax digital T25, IKA-Werke, Germany) for 5 min at 8,000 rpm. For sustained release experiments, B complex vitamins prepared under minimum light exposure to prevent vitamin degradation.

To prepare emulsion containing Ca, sunflower oil (60% v/v), tween 80 (0.05% v/v) and calcium chloride solution 0.1 M (0.05% v/v) were subjected to ultra-sonication at a 24 kHz frequency with 50% of amplitude for 5 min (Hielscher UP400S, Germany). To form a micro gel, 32 ml of emulsion containing Ca was gently added to the 120 ml of VitB- AL–WP emulsion and blended for 20 min at 100 rpm; then 50 ml of calcium chloride solution 0.05 M was added to resulted emulsion. After complete partitioning of droplets to the aqueous phase (about 40 min), white sediment was separated from the creamed oil and they were washed with the solution of calcium chloride 0.05 M and tween 1%. Finally, fabricated AL-WP MGs were filtered using a Millipore glass vacuum filtration system with 0.65 μm cellulose nitrate membranes filter (ALBET). The obtained AL-WP MGs containing vitamin were used immediately to minimize loss of active agent.

AL-WP MGs Characterization

Particle size measurements

The average hydrodynamic diameter and particle size distribution of the AL-WP MGs were determined on fresh diluted samples using dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK). Also, sizes were measured via an optical

microscope equipped with a digital camera; AL-WP MGs diameters estimated were by image J software (version 1.46r).

AL-WP MGs morphology

The microstructure of the dried AL-WP MGs coated using platinum was examined using Leo 1450VP SEM microscope at 5.0 kV. Shape and structure of AL-WP MGs were acquired with Olympus BX41 transmitted light microscope equipped with a Nikon digital camera (Nikon Corp., Tokyo Japan).

ζ-potential measurements

ζ-potential of AL-WP MGs were examined via Malvern Instruments Zetasizer Nano ZS device (Malvern Ltd., UK) using the clear solution of microsphere. All experiment were conducted in three separated injections.

Encapsulation and delivery efficiency

The encapsulation efficiency (EE, %) was determined by dividing the amount of vitamin encapsulated (VE) to the total amount of vitamin (TV) (Zandi, 2017):

$$EE(\%) = \frac{VE}{TV} \times 100 \quad (1)$$

The delivery efficiency (DE) is a capability of the microcapsule to delivery active agent at special condition; this parameter was calculated from the initial (VI) and final (VF) masses of encapsulated vitamin (Zandi, 2017):

$$DE(\%) = \frac{VI-VF}{VI} \times 100 \quad (2)$$

Simulation of gastrointestinal condition

The artificial gastric and intestinal fluids were prepared using Zhang et al. (1981) instruction. The produced simulated intestinal fluid consisted of 10 g of pancreatin and 0.05 mol of potassium dihydrogen phosphate at pH=7. Simulated gastric fluid was prepared by dissolving 2 g of sodium chloride and 3.2 g of pepsin in deionized water at pH=3.

In vitro AL-WP MGs release studies

Release study through the AL-WP MGs shell was investigated at the three different media, including (Zandi, 2017):

Simulated gastric condition

Incubation of 1 g of the wet capsule with 9 ml of the simulated gastric fluid at the 37°C (pH=3) for 150 min with shaking (500 rpm).

Simulated intestinal condition

Incubation of 1 g of the wet capsule with 9 ml of the simulated intestinal fluid at the 37°C (pH=7) for 210 min with shaking (500 rpm).

Simulated gastric-intestinal condition

first, incubation of 1 g of the wet capsule with 9 ml of the simulated gastric fluid at the 37°C (pH=3) for 150 min with shaking (500 rpm), and then added 10 ml of the simulated intestinal fluid to the mixture and incubation the 37°C (pH=7) for 210 min with shaking (500 rpm).

The concentration of the Group B vitamins in the surrounding aqueous phase was measured at various time intervals (30 min) by spectrophotometry method via WPA Lightwave s2000 UV-visible spectro-photometer, (Centerville, VA, U.S.) equipped with a silica cuvette. Sample was filtered through 0.22-lm Biofil syringe filter. Absorbance of final sample at 246 (Ghasemi and Abbasi, 2005), 445 (Chen and Subirade, 2006), 464 (Nwanisobi and Ukoha, 2016), 288 (Khateeb, *et al.*, 2015), 292 (Ghasemi and Abbasi, 2005), 285 (Ghasemi and Abbasi, 2005), 348 (Walash *et al.*, 2008) and 317 (Bruno, 1981) nm were obtained for Thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, folate, biotin and cobalamin, respectively. Standard solution of vitamin and deionized water were used as a calibration sample and zero reference respectively. The AL-WP MGs studies were was repeated three times to verify reproducibility.

AL-WP MGs release Kinetics

In the present research, AL-WP MGs release profile was interpreted with various mathematical models (Dash *et al.*, 2010; Zandi *et al*., 2014)

$$\text{Zero order model: } C_t = C_0 + K_0 t \quad (3)$$

C_t is the amount of vitamin released at time t, C_0 is the initial concentration of d vitamin rug

at time $t = 0$, and K_0 is the zero-order rate constant.

First order model: $\log C_t = \log C_0 - \frac{K_1 t}{2.303}$ (4)

K_1 is the first order rate constant (time^{-1} or per hour).

Korsmeyer -Peppas model:

$$\log(\frac{C_t}{C_\infty}) = \log K_{Kp} + n \log t \quad (5)$$

C_∞ is the amount of vitamin released after time ∞, K_{Kp} is the Korsmeyer release rate constant, and n is the diffusional exponent or drug release exponent.

Kopcha model: $C_t = A \times t^{0.5} + B \times t$ (6)

A and B are the Kopcha constant, and t is the time.

Statistical analysis

Experiments were analyzed using a completely randomized design with repeated measures with the significance level set at $p \leq 0.05$. All statistical analyses and Duncan's post hoc test were carried out at least in triplicate using the SPSS 21.0 statistical software (IBM Corporation, New York City, New York, United States) and graphs' error bars were obtained. All data fittings were accomplished using Matlab software (R2007), and the best model was identified by measuring the correlation coefficient of determination (R^2).

Results and discussion

AL-WP MGs Characterization

Scanning Electron Microscopy (SEM) image obtained for the fabricated AL-WP MGs are depicted in Fig. 1.

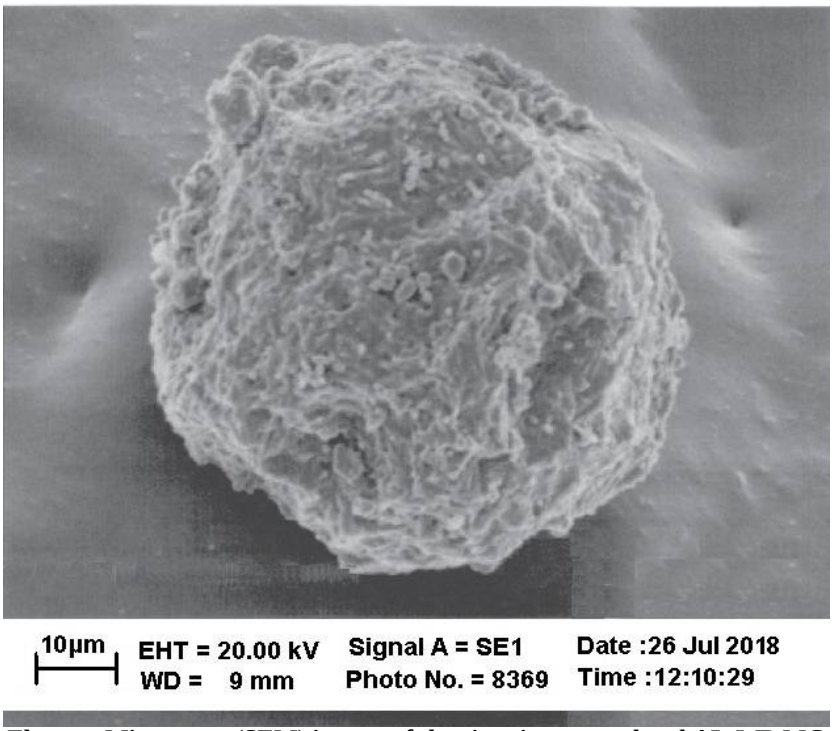

Fig. 1. Scanning Electron Microscope (SEM) images of the vitamin encapsulated AL-WP MGs.

Inspection of images shows that the shape of AL-WP MGs were found to have an almost spherical structure with smoothed and porous shell. This structure probably was developed by the cross-linking of whey protein and alginate by using carboxyl groups (Zandi *et al.*, 2014). Spherical shape was formed due to the exposing of the hydrophilic and hydrophobic the whey protein side chains, respectively, to the solution and core (Zandi, 2017). As shown in Fig. 2,

optical micrograph of AL-WP MGs obtained from light microscopy images confirmed the SEM results. Moreover, it can be seen that most of the resulted AL-WP MGs are under 100 μm in size.

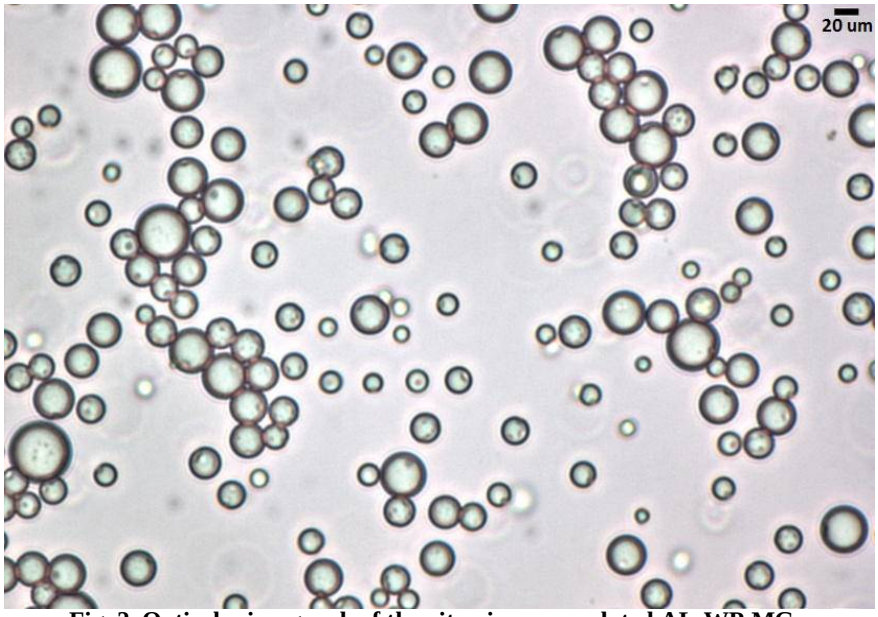

Fig. 2. Optical micrograph of the vitamin encapsulated AL-WP MGs.

The mean diameter of AL-WP MGs were obtained by two different methods. The mean diameter of microcapsule was calculated via image analyzing technique from optical images using ImageJ software (version 1.46r). In this software the equivalent size of AL-WP MGs as the diameter of a circle with equal area were estimated. Image processing results revealed that the diameter of AL-WP MGs range varying between 40–95 μm with an average diameter of 75± 1.3 μm (mean value± SD for n= 50). The size of AL-WP MGs was less than the size range reported by our previous research and other studies (Zandi and Mohebbei, 2015; Zandi, 2017; Zandi *et al*., 2017; Chen and Subirade, 2006). This decreased in the mean diameter might be related to the slight modification the AL-WP MGs fabrication technique and using sonication by ultrasound. This difference confirms that emulsification by ultrasound generally results in average diameters smaller than those obtained with mechanical agitation (Leon *et al*., 2016). By increasing the rate or/ and time of emulsification process, smaller micro gel size can be generated. Particle size distribution curve of AL-WP MGs obtained by dynamic light scattering (DLS) are depicted in Fig. 3. Ity can be seen that the fabricated AL-WP MGs ranging from 35 to 98 μm in size with the mean hydrodynamic diameter 75± 1.3μm. This result has a good correlation with the image processing finding.

The ζ-potential of AL-WP MGs as a function of pH (acidic [gastric] and neutral [intestinal] conditions) were measured.

ζ-potential typically ranges between -100 to +100 mV, and was used to assess the potential stability (Abbasi *et al.*, 2018). For small particles, a higher ζ-potential (negative or positive) will confer stability. So, particles with high ζ-potential are electrically stabilized while particles with low ζ-potential tend to coagulate or flocculate. The ζ-potential values of the AL-WP MGs were abound -68 mV at pH=3 (gastric condition) followed by -14 mV for pH =7 (intestinal situation). These measurements illustrated that the pH had a significant ($P<0.05$) effect on the AL-WP MGs' stability, and these microcapsules had an excellent and moderate

stability in gastric and intestinal condition, respectively. McClements mentioned that multilayered emulsion as a microcapsule have improved stability to environmental stresses than those stabilized by one-layered shell (McClements, 2004; Abbasi *et al.*, 2018).

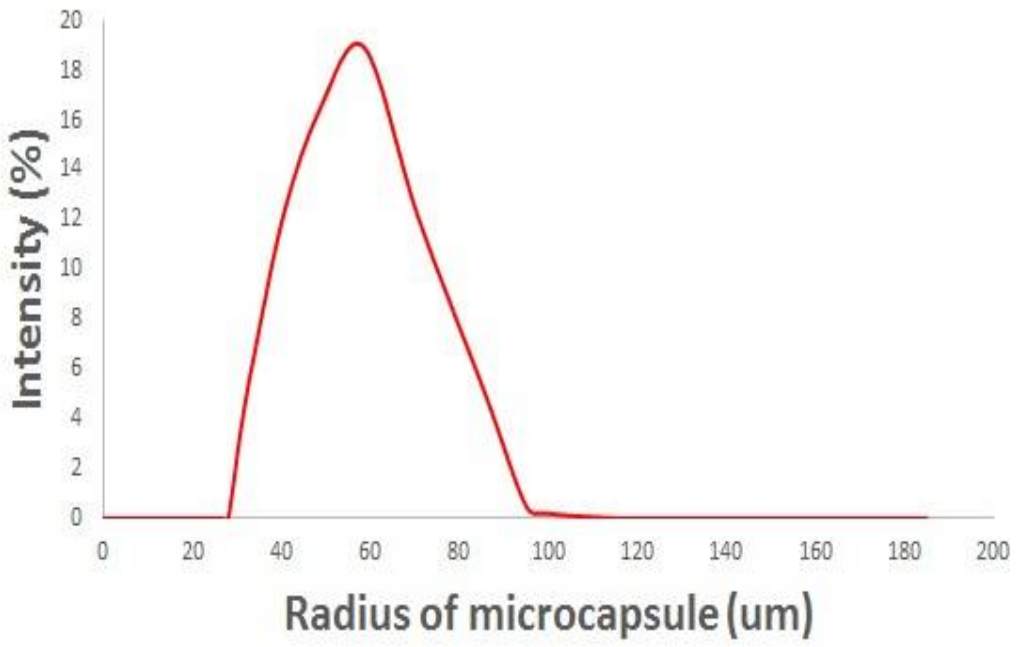

Fig 3. Particle size distribution curve of AL-WP MGs.

Encapsulation efficiency and delivery efficiency of AL-WP MGs

Encapsulation efficiency is often defined as the total amount of vitamin encapsulated in AL- WP MGs with respect to the total amount of the vitamin used. The encapsulation efficiency of AL- WP MGs loaded by thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folate, and cobalamin are shown in Table 1. As seen in Table 1, encapsulation efficiency ranged between 68.89 and 80.46%. Vitamin losses during encapsulation can be affected by the vitamin solubility, sonication time, active agent volatility, microcapsule's shell composite and porosity and emulsifier (Zandi, 2017; Abbasi *et al.*, 2018; Ghorbanzade et al., 2017). Since B complex vitamins are a low molecular weight water-soluble vitamins, its losses during the AL- WP MGs washing step is unavoidable. In current study, lower encapsulation efficiency was obtained for the thiamin encapsulated AL- WP MGs. About 30% of thiamin was lost because of the higher solubility compared to the other B complex vitamins. Results showed that AL-WP MGs contained cobalamin and riboflavin has a higher encapsulation efficiency. Generally it has been reported that the decreasing of solubility and sensitivity, results in better encapsulation efficiency and therefore a greater preservation of bioactive substances.

The Delivery Efficiency (DE) is a capability of the AL-WP MGs to deliver the vitamin at gastric, intestinal and gastric-intestinal conditions (Table 1). As seen in table 1, delivery efficiency ranged between 21.12 and 89.43% for various vitamins and different release situations. It was shown that the delivery efficiency of the AL-WP MGs was higher at simulated gastric-intestinal condition. The lower delivery efficiency reflected a greater resistance to vitamin release. The better delivery efficiency of the vitamin at gastric-intestinal condition is desirable to provide a better protection to the bioactive component in the stomach and a relatively fast release in the intestine.

Table 1. Encapsulation Efficiency (EE) and Delivery Efficiency (DE) of AL-WP MGs loaded by thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folate, and cobalamin.

AL-WP MGs loaded by vitamin	EE (%)	DE at gastric condition (%)	DE at intestinal condition (%)	DE at gastric-intestinal condition (%)
Thiamine	68.89±2.13	21.12±0.89	46.21±1.09	80.25±1.62
Riboflavin	80.46±1.47	26.31±1.12	48.16±1.35	87.74±1.59
Niacin	76.71±1.12	29.11±0.78	50.79±0.72	92.43±0.97
Pantothenic acid	71.64±1.87	23.75±1.24	47.84±1.59	84.74±0.86
Pyridoxine	70.28±1.65	27.98±1.31	48.21±1.42	89.69±1.10
Biotin	73.36±1.04	24.78±0.96	47.25±0.65	82.31±1.45
Folate	69.75±1.59	22.43±1.07	46.34±1.26	79.45±1.72
Cobalamin	77.13±1.56	28.68±0.93	49.57±1.12	88.18±1.32

In Vitro AL-WP MGs Release Studies

In this section, the effects of the release media on the released percentage from the AL-WP MGs was investigated. Vitamin release rate (%min) for various conditions are shown in table 2.

Table 2. Vitamin release rate (%/min) for various conditions

AL-WP MGs loaded by vitamin	release rate (%/min) at various conditions (% ± SD)		
	Gastric condition	Intestinal condition	Gastric-intestinal condition
Thiamine	0.1408±0.013	0.2200±0.012	0.2229±0.009
Riboflavin	0.1754±0.035	0.2290±0.009	0.2437±0.011
Niacin	0.1940±0.024	0.2389±0.010	0.2567±0.015
Pantothenic acid	0.1583±0.015	0.2275±0.008	0.2353±0.013
Pyridoxine	0.1865±0.023	0.2329±0.015	0.2491±0.018
Biotin	0.1652±0.018	0.2243±0.014	0.2286±0.017
Folate	0.1495±0.025	0.2231±0.021	0.2206±0.021
Cobalamin	0.1876±0.019	0.2340±0.013	0.2449±0.011

The in-vitro vitamin release experiments were accomplished in three different simulated conditions, including gastric, intestinal and gastric- intestinal. As expected, release media significantly ($P<0.05$) influenced the vitamin release rate and release profile from AL-WP MGs. This microcapsule showed the highest vitamin release rate at the simulated gastric-intestinal situation. This high release could be due to instability of alginate in neutral pH, and also enzymatic digestion of whey protein. Potent electrostatic interaction between whey protein and alginate in the microcapsule shell caused the stability of AL-WP MGs at the simulated gastric condition (pH=3) (6) (39). Zhang et al. (2016) reported that the protein-polysaccharide interaction depended on the molecular charge of protein and polysaccharide. Three main reasons could find for the WP MGs' stability at the stomach. First constancy of alginate in the acidic media, second, different electrical charge of the whey protein and alginate at acidic conditions and finally, protection effect of the alginate on the whey protein against the gastric enzymes (especially pepsin) via viscosity increasing (Zhang *et al*., 2016; Abbasi *et al*., 2018; Zandi, 2017; Zhang *et al*., 2016; Deat Lainea *et al.*, 2012). Whey protein and alginate strongly have tended to attract and repel each other at acidic and neutral pH, respectively. Therefore, AL-WP MGs at the neutral pH (i.e. intestinal condition) probably had an open structure with more and larger pores. This structure may be responsible for the faster release in the simulated intestinal condition (Zhang *et al.*, 2016). Our release results is in agreement with the pervious researches (Zandi, 2017) (Zhang, *et al.*, 2015) (Chen and Subirade, 2006). It was found that type of the vitamin had a slighter effect on the release rate and release profile. The result indicated that vitamin release rate

was increased with increases vitamin solubility.

Fig. 4 shows the typical profile of vitamin release from AL-WP MGs (for biotin). The release profiles were built by plotting the cumulative vitamin release percentile versus the release time. As clearly seen, the vitamin release profile has a two curve with the different slope. First, quick burst releases, and then a slow diffusion starts to release. Rapid release mainly occurs from holes and pores, and slow release corresponded to the diffusion mechanism through the AL-WP MGs' shell.

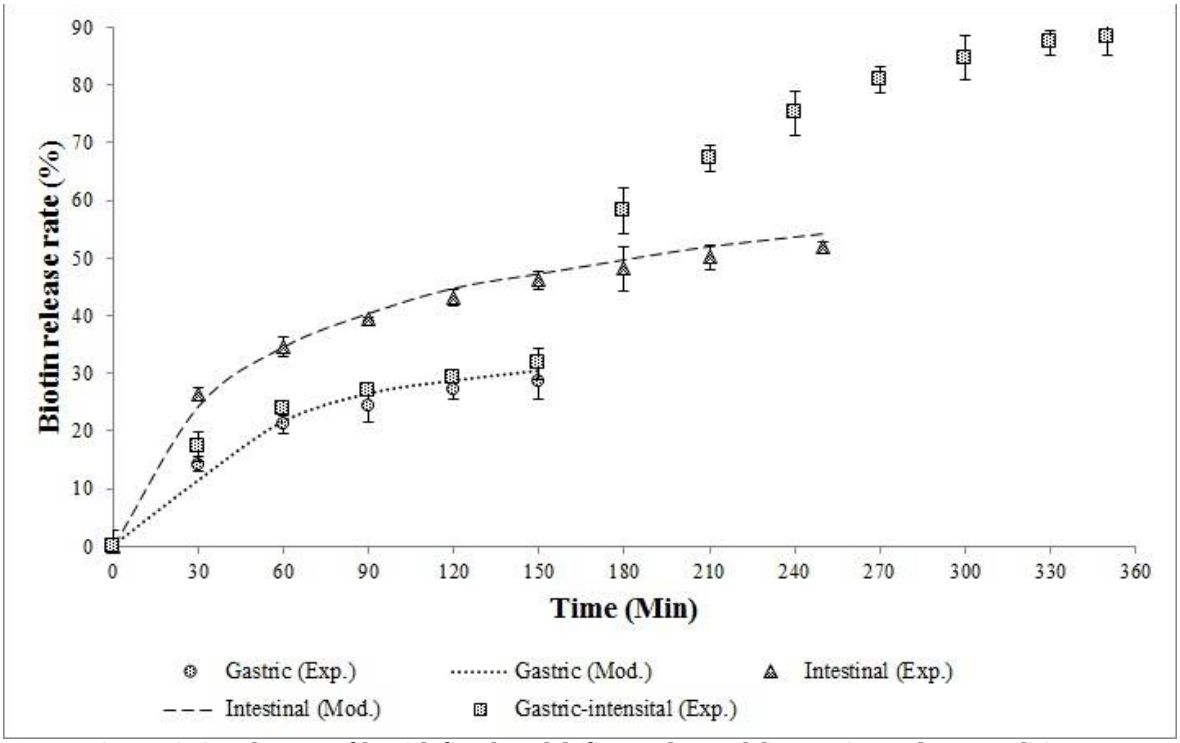

Fig. 4. Biotin release profile with fitted model (first-order model) at various release conditions.

Mathematical modeling for vitamin release kinetics

To investigate the vitamin release from AL-WP MGs at various situations, mathematical modeling was accomplished via various kinetic equations (including Zero Order model, First order model, Korsmeyer –Peppas model, and kopcha model) (Table 3, 4, 5). These kinetic equations were used to the vitamin release mechanism recognition, release rate prediction, and vitamin release physics understanding. For this purpose, experimental release data were fitted to the various kinetics models, and the best one was selected according to the regression coefficient evaluation. As seen, vitamin release profile was non-linear and doesn't follow zero-order model (R^2 between 21.43-36.72 in Table 3, 4, and 5).

The modeling results indicated that the first-order model could be the best describe for group B vitamins with R^2 between 97.43-99.15. However, the other mathematical model that best described vitamin releases from AL-WP MGs were Korsmeyer– Peppas model and kopcha model with R^2 values greater than 0.842. As observed in Table 4, the Korsmeyer–Peppas release exponent (n) ranged between 0.1014- 0.4313 which confirms that fickian diffusional release is the main mechanism. n is the diffusional exponent or drug release exponent. Hence, n value is used to characterize different release mechanisms; when the Korsmeyer– Peppas release exponent

is less than 0.5, Fickian diffusion is the main mechanism for vitamin release. For more information about release mechanism, kopcha model was used. In this kinetic model, A and B are diffusional and erosional terms respectively. When A/B ratio is greater than 1, then fickian diffusional is the main mechanism of release. For this purepose must be A component far greater than B component. As seen in Table 4, the Korsmeyer– Peppas and Kopcha models suggested that release from group B vitamins mainly was controlled by Fickian diffusion mechanism.

Table 3. Results of model fitting of vitamin release from AL-WP MGs in simulated gastric condition

AL-WP MGs loaded by vitamin	Kinetic Models									
	Zero order		First order		Korsmeyer -Peppas			Kopcha		
	K_0	R^2	K_1	R^2	K_{Kp}	n	R^2	A	B	R^2
Thiamine	0.2312	25.36	0.0831	97.83	0.2653	0.1543	89.43	0.1998	-0.0231	95.93
Riboflavin	0.1321	27.85	0.1284	97.43	0.3214	0.2115	88.65	0.2111	-0.0344	97.15
Niacin	0.1432	21.43	0.1543	97.54	0.4321	0.2419	89.93	0.2243	-0.0451	96.49
Pantothenic acid	0.2127	29.43	0.1321	98.29	0.4215	0.1126	90.21	0.2831	-0.0387	98.54
Pyridoxine	0.2657	25.68	0.1654	99.08	0.3614	0.2078	89.67	0.2567	-0.0421	97.73
Biotin	0.2981	34.58	0.1023	98.43	0.3812	0.2012	91.12	0.2113	-0.0426	98.15
Folate	0.3021	36.71	0.0976	98.45	0.3314	0.2923	90.65	0.2017	-0.0349	96.31
Cobalamin	0.3012	35.98	0.1215	99.01	0.4341	0.2877	91.48	0.2165	-0.0409	98.11

Table 4. Results of model fitting of vitamin release from AL-WP MGs in simulated intestinal condition.

AL-WP MGs loaded by vitamin	Kinetic Models									
	Zero order		First order		Korsmeyer -Peppas			Kopcha		
	K_0	R^2	K_1	R^2	K_{Kp}	n	R^2	A	B	R^2
Thiamine	0.2921	28.45	0.1342	98.24	0.3654	0.2384	89.12	0.3123	-0.0317	97.47
Riboflavin	0.3021	29.41	0.1541	99.04	0.4532	0.3876	88.96	0.3651	-0.0288	98.30
Niacin	0.2121	32.31	0.1532	97.48	0.4431	0.2487	90.91	0.4567	-0.0412	98.57
Pantothenic acid	0.2541	35.45	0.1245	99.11	0.4832	0.3421	90.24	0.3217	-0.0406	97.26
Pyridoxine	0.2632	33.21	0.2341	98.67	0.4211	0.2987	90.65	0.4213	-0.0321	99.01
Biotin	0.2147	34.47	0.2126	97.19	0.3641	0.3218	89.36	0.2987	-0.0504	98.91
Folate	0.3076	29.93	0.2376	99.10	0.3523	0.3991	88.67	0.2876	-0.0419	98.40
Cobalamin	0.2431	28.54	0.2020	98.95	0.3971	0.3772	91.24	0.4965	-0.0287	98.75

Table 5. Results of model fitting of vitamin release from AL-WP MGs in simulated gastric-intestinal condition

AL-WP MGs loaded by vitamin	Kinetic Models									
	Zero order		First order		Korsmeyer -Peppas			Kopcha		
	K_0	R^2	K_1	R^2	K_{Kp}	n	R^2	A	B	R^2
Thiamine	0.2312	28.45	0.2851	98.76	0.4123	0.3217	89.67	0.4832	-0.0501	95.90
Riboflavin	0.2465	39.31	0.4321	99.15	0.4982	0.4313	90.54	0.4751	-0.0365	98.74
Niacin	0.4031	30.12	0.1243	98.45	0.5321	0.4215	90.36	0.4231	-0.0287	97.96
Pantothenic acid	0.3126	28.98	0.2356	99.01	0.5412	0.3254	91.11	0.4034	-0.0391	98.52
Pyridoxine	0.3216	34.23	0.2945	97.68	0.4321	0.3765	92.35	0.3657	-0.0402	98.01
Biotin	0.3021	35.59	0.2542	98.24	0.3987	0.3821	91.70	0.3987	-0.0294	97.45
Folate	0.2187	31.23	0.2098	98.99	0.4534	0.3954	92.16	0.3765	-0.0367	98.17
Cobalamin	0.3476	32.91	0.4231	98.43	0.4673	0.4212	90.07	0.4112	-0.0299	98.50

Conclusion

The focus of this work was to produce water-in-oil emulsion stabilized by whey protein and alginate to protect vitamin. Investigation of SEM image indicated that the shape of fabricated AL-WP MGs were found to have an almost spherical structure with an average diameter of 75± 1.3μm. The ζ-potential measurements illustrated that the pH had a significant (P<0.05) effect on the AL-WP MGs'

stability. Accordingly, this microcapsule showed the highest vitamin release rate at the simulated gastric-intestinal situation. This high release could be due to instability of alginate in neutral pH, and also enzymatic digestion of whey protein. The results indicated that fickian diffusional release is the main mechanism for group B vitamins from AL-WP MGs. These micro gel therefore appears to be potentially beneficial as digestion delivery vehicles for bioactive compounds in the food and nutraceuticals industry as well as non-food industry.

Declaration of interest

This work was supported by the Iran National Elites Foundation. Financial support of this research by National Elites Foundation through a contract with the University of Zanjan (Iran) is gratefully acknowledged.

Reference

Abbasi, F, F Samadi, S M Jafari, S Ramezanpour, and M S Shargh. 2018. "Ultrasound-assisted preparation of flaxseed oil nanoemulsions coated with alginate-whey protein for targeted delivery of omega-3 fatty acids into the lower sections of gastrointestinal tract to enrich broiler meat." *Ultrasonics Sonochemistry*.

Beck, W S. 2001. *In Handbook of Vitamins, 3rd edn ed*. Edited by R Rucker, J Sutie, D B McCormick and L J Machlin. New York: Marcell Dekker.

Bouyer, E, G Mekhloufi, V Rosilio, J L Grossiord, and F Agnely. 2012. "Proteins, polysaccharides, and their complexes used as stabilizers for emulsions: Alternatives to synthetic surfactants in the pharmaceutical field?" *International Journal of Pharm* 436: 359– 378.

Bruno, P. 1981. "A New Spectrophotometric Method for Determination of Vitamin B12 as Cobalts." *Analytical Letters* 14, (18): 1493-1500.

Chen, L., Subirade, M. 2006. "Alginate–whey protein granular microspheres as oral delivery vehicles for bioactive compounds." *Biomaterials* 27 : 4646–4654.

Cheong, A M, C P Tan, and K L Nyam. 2016. "In-vitro gastrointestinal digestion of kenaf seed oil-in-water nanoemulsions." *Indinia Crops Production* 87: 1-8.

Ching, Su Hung , Nidhi Bansal , and Bhesh Bhandari. 2017. "Alginate gel particles–A review of production techniques and physical properties." *Critical Reviews in Food Science and Nutrition* 57 (6): 1133-1152.

Dash, S, P N Murthy, L Nath, and P Chowdhury. 2010. "Kinetic modeling on drug release from controlled drug delivery systems." *Acta Pol Pharm* 67: 217-223.

Déat-Lainéa, E, V Hoffarta, J M Cardota, M Subiradeb, and E Beyssaca. 2012. "Development and in vitro characterization of insulin loaded whey protein and alginate microparticles,. 439 (2012) ." *International Journal of Pharmaceutical* 439: 136–144.

Fani, A A, R S Fortuny, and O M Belloso. 2017. "Nanoemulsions as edible coatings." *Current Opinion Food Science* 15: 43-49.

Ghasemi, Jahanbakhsh , and Bahman Abbasi. 2005. "Simultaneous Spectrophotometric Determination of Group B Vitamins Using Parallel Factor Analysis: PARAFAC." *Journal of the Chinese Chemical Society* 52: 1123-1129.

Ghorbanzade, T, S M Jafari, S Akhavan, and R Hadavi. 2017. "Nano-encapsulation of fish oil in nano-liposomes and its application in fortification of yogurt." *Food Chemistry* 216: 146-152.

Jafari, S M. 2017. *Nanoencapsulation of food bioactive ingredients.* Elsevier.

—. 2017. *Nanoencapsulation technologies for the food and nutraceutical industries.* Elsevier.

Khateeb , Mouhammed , Basheer Elias, and Fatema AL Rahal. 2015. "Validated Spectrophotometric Method to Assay of B6 and B3 Vitamins in Pharmaceutical Forms Using Potassium Iodide and Potassium Iodate." *International Letters of Chemistry, Physics and Astronomy* 60: 113-119.

Koutina, G, C A Ray, R Lametsch , and R Ipsen. 2018. "The effect of protein-to-alginate ratio on in vitro gastric digestion of nanoparticulated whey protein." *International Dairy Journal* 77: 10-18.

Lamas, M C, C Bregni, M D Aquino, J Degrossi, and R Firenstein. 2001. "Calcium Alginate Microspheres of Bacillus subtilis." *Drug Dev, Ind. Pharm* 27: 825–829.

LeBlanc, J G, J E Lain, M Juarez del Valle, V Vannini, D van Sinderen, M P Taranto, G Font de Valaz, G Savoy de Giori , and F Sesma. 2011. "B-Group vitamin production by lactic acid bacteria – current knowledge and potential applications." *Journal of Applied Microbiology* 111: 1297-1309.

Leon , A M, W T Medina, D J Park, and J M Aguilera. 2016. "Mechanical properties of whey protein/Na alginate gel microparticles." *Journal of Food Engineering* 188: 1-7.

Leon, Alicia M , Wenceslao T Medina, Dong J Park , and Jose´ M Aguilera. 2018. "Properties of microparticles from a whey protein isolate/alginate emulsion gel." *Food Science and Technology International 0(0) 1–10* accepted: 1-10.

McClements, D J. 2015. "Encapsulation, protection, and release of hydrophilic active components: Potential and limitations of colloidal delivery systems." *Advances in Colloid and Interface Science* 219: 27-53.

McClements, D J. 2004. "Protein-stabilized emulsions." *Current Opinon Colloid Interface Science* 9: 305-311.

Molina, V C, M Medici, M P Taranto, Font de Valdez, and G Font de Valdez. 2009. "Lactobacillus reuteri CRL 1098 prevents side effects produced by a nutritional vitamin B deficiency." *Journal of Applied Microbiology* 106: 467–473.

Moll, Rachel, and Bernard Davis. 2017. "Iron, vitamin B12 and folate." *Medicine* 45 (4): 198-203.

Moschona, Alexandra , and Maria Liakopoulou-Kyriakides. 2018. "Encapsulation of biological active phenolic compounds extracted from wine wastes in alginate-chitosan microbeads." *Journal of Microencapsulation* accepted: 1-36.

Ni, Y, L Wen, L Wang, Y Dang, P Zhou, and L Liang. 2015. "Effect of temperature, calcium and protein concentration on aggregation of whey protein isolate: Formation of gellike micro-particles." *International Dairy Journal* 51: 8–15.

Nwanisobi , G C, and P O Ukoha. 2016. "Spectrophotometric Determination of Niacin Using 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone." *Asian Journal of Chemistry* 28 (11): 2371-2374.

Oehlke, K, M Adamiuk, D Behsnilian, V Graf, E Mayer-Miebach, E Walz, and R Greiner. 2014. "Potential bioavailability enhancement of bioactive compounds using food-grade engineered nanomaterials: a review of the existing evidence." *Food & Function* 5 (7): 1341-1359.

Walash, M I, M Rizk, Z A Sheribah, and M M Salim. 2008. "Kinetic Spectrophotometric Determination of Biotin in Pharmaceutical Preparations." *International journal of Biomedical science* 4 (3): 238-244.

Wichchukit, S, M H Oztop, M J McCarthy, and K L McCarthy. 2013. "Whey protein/alginate beads as carriers of a bioactive component." *Food Hydrocolloids* 33: 66-73.

Zandi , M, N Dardmeh, S Pirsa, and H Almasi. 2017. "Identification of Cardamom Encapsulated Alginate–Whey Protein Concentrates Microcapsule Release Kinetics and Mechanism during Storage, Stew Process and Oral Consumption." *Journal of Food Process Engineering* 40 (1): 1-9.

Zandi, M., Mohebbi, M. 2015. "An agent-based simulation of a release process for encapsulated flavor using NetLogo platform." *Flavor and Fragnace Journal* 30: 224–229.

Zandi, M., Mohebbi, M. 2014. "Investigation of encapsulated diacetyl colloidosome release profile as a function of sintering process and release media properties." *Flavor and Fragnace Journal* 29 (6): 364-370.

Zandi, M., Mohebbi, M., Varidi, M., Ramezanian, N. 2014. "Evaluation of diacetyl encapsulated alginate-whey protein microspheres release kinetics and mechanism at simulated mouth conditions." *Food Research International journal* 56: 211-217.

Zandi, M., Pirsa, S., Dardmeh, N. 2017. "Simulation of ascorbic acid release from alginate-whey protein concentrates microspheres at the simulated gastro-intestinal condition using NetLogo platform." *Journal of Food Process Engineering* 40 (1): 1-9.

Zandi, Mohsen. 2017. "Evaluation of the kinetics of ascorbic acid (aa) release from alginate-whey protein concentrates (al-wpc) microspheres at the simulated gastro–intestinal condition." *Journal of Food Process Engineering* 40 (1): 1-11.

Zhang, Z, R Zhang, L Chen, Q Tong, and D J McClements. 2015. "Designing hydrogel particles for controlled or targeted release of lipophilic bioactive agents in the gastrointestinal tract." *European Polymer Journal* 72: 698-716.

Zhang, Zipei , Ruojie Zhang, Liqiang Zou, and David Julian McClements. 2016. "Protein encapsulation in alginate hydrogel beads: Effect of pH on microgel stability, protein retention and protein release." *Food Hydrocolloids* Accepted: 1-30.

Zhang, Y, Q C Wang, H Yu, J Zhu, K de Lange, Y Yin, Q Wang, and J Gong. 2016. "Evaluation of alginate–whey protein microcapsules for intestinal delivery of lipophilic compounds in pigs." *Journal of Science Food and Agricultur* 96: 2674–2681.

Iranian Food Science and Technology Research Journal
Vol. 16, No. 3, Aug. Sep. 2020, p. 37-50

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد 16، شماره 3، مرداد - شهریور 1399، ص. 50-37

محافظت ویتامین از شرایط سیستم گوارش با استفاده از میکروژل آلژینات- پروتئین آب پنیر. مطالعه موردی ویتامین B کمپلکس

محسن زندی*

تاریخ دریافت: 1397/12/04
تاریخ پذیرش: 1398/06/10

چکیده

کمبود ویتامین اخیراً در برخی از کشورها به سبب رژیم غذایی نامتعادل یا ناقص وجود دارد، از این‌رو غنی‌سازی مواد غذایی با ویتامین ضروری می‌باشد. محافظت ویتامین در میکروژل سبب افزایش پایداری و زیست فراهمی عوامل فعال در برابر شرایط سیستم گوارش می‌گردد. هدف تحقیق اخیر تعیین، مقایسه و توسعه سیستم تحویل ایده‌آل به‌منظور محافظت ویتامین در برابر شرایط گوارش می‌باشد. برای این منظور، میکروژل آلژینات- پروتئین آب پنیر حاوی ویتامین به‌عنوان حامل بیوپلیمری ایجاد و توسعه یافت. این میکروکپسول از منظر مورفولوژی، اندازه‌گیری پتانسیل زتا، اندازه‌گیری توزیع اندازه ذرات، راندمان انکپسولاسیون و تحویل و در نهایت هضم در شرایط روده و معده آزمایشگاهی مورد آزمایش قرار گرفت. روش جذب برای کنترل رهایش ویتامین B در شرایط معده در طول مدت آزادسازی مورد استفاده قرار گرفت. آزمون‌های رهایش ویژگی‌های مفیدی را برای این نوع میکروکپسول مشخص نمود. مکانیسم رهایش با استفاده از مدل‌های سینتیکی پیش‌بینی گردید. نتایج نشاندهنده این بود که اکثر میکروکپسول‌ها به‌صورت کروی با اندازه 100 میکرومتر می‌باشد و این میکروکپسول‌ها به‌ترتیب دارای پایداری بسیار خوب و متوسط در شرایط معده و روده هستند. نتایج همچنین نشان داد که بیشترین میزان رهایش در شرایط معده- روده رخ داده و نوع ویتامین تاثیر اندکی بر میزان رهایش و پروفایل رهایش دارد. مدل‌های سنتیکی پیشنهاد می‌دهد که رهایش ویتامین‌های خانواده B عمدتاً با مکانیسم فیک دیفوزیون رخ می‌دهد. به‌طور کلی، این تحقیق نشان داد که میکروژل آلژینات- پروتئین آب پنیر حاوی ویتامین می‌تواند ویتامین را در برابر هضم معدوی محافظت نموده و به‌عنوان سیستم تحویل استفاده گردد.

واژه‌های کلیدی: ویتامین B کمپلکس، رهایش کنترل شده، میکروژل، پروتئین آب پنیر، آلژینات

1- استادیار گروه علوم و مهندسی صنایع غذایی، دانشگاه زنجان، دانشکده کشاورزی، گروه علوم و مهندسی صنایع غذائی.
(*- نویسنده مسئول: Email: Zandi@znu.ac.ir)