

Comparison of in vitro release profile of betaine from a multi-layered and single-layered electrospun structure based on Plantago ovata seed gum under the simulated digestive system

Zahra Abdollahzadeh

Islamic Azad University Science and Research Branch

Masoud Honarvar

m-honarvar@hotmail.com

Islamic Azad University Science and Research Branch

Mehrdad Ghavami

Islamic Azad University Science and Research Branch

Research Article

Keywords: Molasses, Betaine, Electrospinning, Fast-dissolving, Plantago ovata seed gum, sandwich structure

Posted Date: December 11th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3632156/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Waste and Biomass Valorization on March 25th, 2024. See the published version at <https://doi.org/10.1007/s12649-024-02477-5>.

Abstract

This study aimed to extract aspartic acid from sugar beet molasses using the cloud point method and encapsulate it in the structure of electrospun fast-dissolving poly(vinyl alcohol) (PVA)/*Plantago ovata* (*P. ovata*) seed gum fibers as the central layer of the sandwich structure. The outer layers of the sandwich structure were designed with corn zein electrospun fibers. The field emission scanning electron microscopy showed that the concentration of 35% w/v zein in 70% v/v ethanol provides uniform electrospun fibers for use as a delayed hydrophobic layer in a sandwich structure. The contact angle of the sandwich structure (92.45°) was significantly ($p < 0.05$) higher than the contact angle of electrospun mats of PVA/*P. ovata* seed gum containing 10% w/v sugar beet molasses betaine (53.5°). Fourier transform infrared spectroscopy and differential scanning calorimetry showed that the encapsulation process was successful. The use of a sandwich layer compared to the electrospun single-layer structure of PVA/*P. ovata* seed gum led to an increase in the thermal stability of betaine. *In vitro* release modeling showed that for all the structures of the Peppas-Sahlin model, it was the best model to describe the release behavior, and the Fickian diffusion mechanism was the dominant effective mechanism in betaine release.

Introduction

Sugar beet was introduced as the first source of betaine glycine (N,N,N-Trimethylglycine), which is known as a zwitterionic ammonium ion compound. The methyl groups present in the structure of betaine have caused it to be considered an essential source of this group in methyl transfer reactions. By converting homocysteine to methionine, betaine reduces the risk of heart diseases in the human body, which it does through its methyl-donating ability. Homocysteine is a cause of cardiovascular disease that can be prevented by taking methyl groups in betaine. Getting methyl groups through diet is necessary because the body cannot synthesize them, so the body needs to receive them continuously [1, 2]. Therefore, sugar beet and the waste resulting from its processing, especially molasses, can be considered a potential source of this valuable compound. Molasses is considered waste from the process of extracting sugar from sugar beets, which is 4–5% of beets [3, 4]. The low price of this by-product can be considered a valuable source of bioactive and nutraceutical compounds for food and pharmaceutical industry researchers. Therefore, using new extraction methods to extract its valuable compounds, especially betaine, can be an excellent potential for producing products with high added value from this wastewater [5].

The electrospinning process, as one of the novel delivery methods for encapsulation and targeted delivery of bioactive compounds, has received much attention in recent years. The electrospinning process, which consists of four main parts, pump, high voltage power supply, needle, and collector, leads to the production of spun fibers with unique characteristics with a high surface-to-volume ratio. Micron-, submicron-, and nano-scale of these electrospun fibers with high porosity, amorphous state, and acceptable mechanical properties will be valuable for many nutraceutical delivery applications [6, 7]. A convenient process that can be performed at ambient temperature will create a very valuable potential for

heat-sensitive compounds that are vulnerable to inappropriate process conditions [8]. Electrospun fibers can be used as a carrier for many bioactive and nutraceutical compounds such as vitamins [9], nanoparticles [10], essential oils [11], lycopene [12], [13], bergamot [8], bacteriocins [14], etc.

The use of hydrophilic polymers and biopolymers in the electrospinning process will lead to the production of superhydrophilic and fast-dissolving structures that offer the valuable potential for delivering water-insoluble compounds and nutraceutical compounds to the mouth for lingual absorption. Also, to be used in food products, they require fast and immediate release of aroma and taste compounds [8]. However, these fast-dissolving structures can create many limitations for the targeted delivery of bioactive compounds. Therefore, the use of bio-engineering methods can solve some of these limitations. Using multilayer structures that combine hydrophilic-hydrophilic or hydrophilic-hydrophobic polymers can be a good potential to solve some limitations [7, 12]. Polysaccharides and gums are hydrophilic compounds whose electrospinning process is associated with the production of fast-dissolving fibers and particles [15]. Our study has shown that because of its hydrophilic nature, *P. ovata* seed gum can produce fast-dissolving electrospun fibers [16]. Also, this ability to fast-dissolving has been reported in other studies for different gums, which delayed the release of bioactive compounds loaded in them by using sandwich structures and three layers. Rezaeinia et al. (2020) designed fast-dissolving structures based on menthol-loaded gelatin that released 85% of menthol within 25 s. But by using sandwiching layers based on Balangu seed gum/poly(vinyl alcohol) (PVA) on the central layer of gelatin containing menthol, led to the release of 83% of menthol within 55 s [8]. Also, Kazemianrad et al. (2023) studied electrospun nanofibers based on caffeine-loaded *Alyssum homolocarpum* seed gum to produce three-layer structures. They reported that more than 50% of caffeine was released from the monolayer structures in less than 60 s. However, using electrospun gelatin fibers as the outer layers of the sandwich structure led to an increase in the release time of caffeine in the simulated media of human saliva [13].

Therefore, the purpose of this research is to design a sandwich structure consisting of *P. ovata* seed gum/PVA loaded with betaine extracted from sugar beet molasses as the central layer. Also, the outer layers of the sandwich structure are made of electrospun fibers of corn zein biopolymer. The release process of betaine extracted from molasses from monolayer structures and sandwich structures is evaluated in the simulated media of mouth, stomach, and intestine. Also, the characteristics of electrospun fibers loaded with betaine are assessed in terms of morphological, thermal, and FTIR characteristics.

Experimental

Material and methods

P. ovata seeds were obtained from the local market in Tehran, Iran. Corn zein (purity > 98%), ethanol, HCl, NaOH, and CaCl₂ were purchased from Merck Co., (Germany). Also, bile salts, pancreatic solution, and pepsin enzyme were obtained from the Sigma-Aldrich Co. (USA).

Betaine extraction from molasses

The cloud point extraction method was used to extract betaine from sugar beet molasses, and the details of the extraction method were fully described in our previous study [16].

Extraction of the gum of the saffron seed

After grinding the *P. ovata* seeds, using a 30-mesh sieve, the husks were separated from the kernels and mixed with a distiller at a ratio of 1:30. After overnight, the gum was separated from the pulp using a centrifuge for 15 min at 5000 rpm. Finally, the resulting gum was dried at room temperature after purification with ethanol [17].

Electrospinning of *P. ovata* seed gum

During our previous study, the electrospinning process of *P. ovata* seed gum was thoroughly studied and evaluated. According to the rheological properties of the electrospun feed solutions as well as the morphological and thermal properties of the electrospun fibers, it was determined that the ratio of 6 to 4 (v/v) for PVA to *P. ovata* seed gum was selected as the optimal sample and betaine were loaded in its structure. Based on this, during this research, this ratio of PVA to *P. ovata* seed gum was used to produce sandwich and single-layer structures. For this purpose, a 10% solution of PVA and a 1% solution of *P. ovata* seed gum were prepared and mixed at a ratio of 6 to 4 v/v, and betaine extracted from molasses with a concentration of 10% w/v was added to the above mixture, and spinning was performed. The spinning operation was performed using a syringe pump (Mdeifusion MS 2200, South Korea) with a flow rate of 1.5 ml/h, an electric field with a voltage of 25 kV, and a distance between the tip of the needle and the collector (stainless steel 50 × 50 mm²) of 150 mm [16].

Production of sandwich structure

To produce a sandwich structure, a hybrid mat of *P. ovata* seed gum/PVA loaded with betaine was chosen as the center layer. Also, pure zein solution with concentrations of 30, 35, and 40% w/v in 70% v/v ethanol solution was selected as the outer layers of the sandwich structure. For this purpose, first, each of the solutions was spun in the electrospinning process (pump flow rate 0.5 ml/h, the distance between the collector and the needle tip 150 mm, and the electric field with a voltage of 25 kV) to produce uniform and defect-free fibers with the smallest diameter. After that, the uniform electrospun fibers of zein were selected using FESEM images. Finally, the desired concentration of zein was used to sandwich the central layer loaded with betaine. For this purpose, first, the outer layer was spun on the collector for 15 min, then the central layer consisting of *P. ovata* seed gum/PVA containing betaine was placed on the first layer for 30 min. Finally, the third layer composed of pure zein was placed on the other two layers for 15 min under constant conditions similar to the first layer [7].

Morphological characteristics

Morphological properties of zein electrospun solutions with concentrations of 30, 35 and 40% and electrospun hybrid fibers of *P. ovata* seed gum/PVA were evaluated using field emission scanning

electron microscopy (FESEM, MIRA3, TESCAN, Czech Republic). For this purpose, each of the electrospun fibers was first covered by a thin layer of gold by a sputter coater (Quorum Technologies Ltd., UK) [11].

Contact angle

To determine the hydrophilicity or hydrophobicity of each produced sample, the contact angle of the water droplet with the surface of electrospun samples loaded with betaine was used. For this purpose, a drop of distilled water will be dripped on the surface of each sample using a micropipette (Eppendorf Research® Plus Single Channel Pipette), and a high-speed digital camera (Dino-Lite AM3113 digital, USA) will be used to photograph the moment the drop touches the surface. Then the obtained images were analyzed in the Image-Pro Plus software (Version 7.3), and the contact angle of the droplet with the surface was determined [18].

Fourier transform infrared (FTIR) analysis

The possibility of reaction between the constituents of hybrid mats and loaded betaine, as well as hybrid mats with sandwiching zein electrospun fibers, was investigated using the Fourier transform infrared spectroscopy (Bruker Alpha FTIR, US) test in the wave number range of 4000 to 400 cm^{-1} [19].

Differential scanning calorimetry (DSC) analysis

The thermal stability of betaine and the confirmation of the successful encapsulation of betaine in the designed structures were tested using differential scanning calorimetry (DSC, IRSA DSC100, Iran). Electrospun samples and pure betaine were heated in the thermal range of 25 to 400°C with a weight of 15 mg and a temperature rate of 10°C/min. Using the resulting thermograms, the state of betaine encapsulation was compared with its pure sample [20].

In vitro betaine release modeling from designed structures

The modeling of betaine release from the single-layer structures of *P. ovata* seed gum/PVA hybrid mats and the three-layer sandwich structure was evaluated in the *in vitro* simulated media. For this purpose, according to the fast-dissolving nature of *P. ovata* seed gum/PVA hybrid mats loaded with betaine, betaine release modeling was evaluated from the structures only in human saliva simulation medium (25 mM KH_2PO_4 with pH = 6.8). However, the sandwich structure of its release during three different simulated oral, gastric and intestinal phases was investigated [7].

Release analysis was performed in the oral phase under stirring conditions of 100 rpm at a temperature of 37°C for 10 min with different time intervals (for the first minute every 5 s, and in the second minute every 30 s and for the remaining 8 minutes every 1 min).

The method recommended by Sarkar et al. (2009) to investigate the betaine release process from the designed structures in the simulated phase of the stomach (7 ml of 37% HCl solution with 2 g of NaCl and 2.3 g of pepsin enzyme in 1 L of water were dissolved, pH = 1.2) was used.

The simulated intestinal phase was prepared using 0.25 M calcium chloride solution and 3.75 M sodium chloride solution with pH = 7. For this purpose, the pancreatic solution with a concentration of 16.25 mg/ml, lipase solution with a concentration of 24 mg/ml, and bile salts with a concentration of 46.87 mg/ml were used [21].

The release analysis was performed in the simulated oral phase for the betaine-loaded *P. ovata* seed gum/PVA hybrid samples. But for the sandwich samples, 20 ml of the sample treated with the oral phase solution was mixed with 20 ml of simulated gastric solution. The pH was adjusted to 2.5 with 1 M sodium hydroxide solution. Next, the resulting solution was placed at a temperature of 37°C with 100 rpm for 2 h. Sampling of this phase was done at different intervals of 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, and 120 min. Next, 30 ml of the above solution was stirred at 37°C at 100 rpm, 3.5 ml of bile salts solution was added to it and the pH was adjusted to 7. Then 2.5 ml of lipase solution and 3 ml of pancreatic solution were added to the mixture and placed at 37°C for 2 h. Sampling was done at different intervals of 2, 5, 10, 20, 40, 80, 120, 150, 180, 210, 240, 270, 300, 330, and 360 min [7].

For each phases, the absorbance of each sample obtained from different time intervals was measured using a spectrophotometer (UNICO-2100, USA) at 365 nm, and the amount of released betaine was determined using the betaine standard curve. Finally, the release process of betaine under simulated conditions was fitted using different mathematical models (Higuchi, Rigter-Peppas, Peppas-Sahlin, and Kopcha), and the main mechanism of betaine release and mass transfer was determined in simulated media.

$$M_t/M_\infty = kt^{1/2} \text{ Eq. 1 [22]}$$

$$M_t/M_\infty = kt^n \text{ Eq. 2 [23]}$$

$$M_t/M_\infty = k_1 t^m + k_2 t^{2m} \text{ Eq. 3 [24]}$$

$$M_t = Axt^{0.5} + Bxt \text{ Eq. 4 [25]}$$

Where M_t is the amount of betaine at time t , M_∞ is the amount of betaine released at the end of the test, k is the release constant, and t is the test time. The n is the release exponent, and in cylindrical-like structures $n < 0.45$, it indicates the dominant mechanism of Fickian diffusion. The k_1 , k_2 , and m are the Fickian diffusion constant, erosion constant, and Fickian diffusion exponent, respectively. If the ratio $k_1/k_2 > 1$, the dominant mechanism is mainly of the Fickian diffusion type. The A is the Fickian release constant, and B is the erosion constant. If $A/B > 1$, the dominant mechanism is the Fickian diffusion type. Root mean square error (RMSE) and coefficient of explanation (R^2) indices are used to select the best model describing betaine release behavior from the designed structures. $RMSE < 10$ indicates an excellent fit of the data with the proposed model, and as the RMSE exceeds 10, the goodness of fit decreases [26].

Statistical analysis

One-way ANOVA was used to check the significant difference between the data using the IBM SPSS statistics software (version 22.0, IBM Corp., USA). Duncan's multiple range test was used at the 95% confidence level to compare the average treatments. In addition, MATLAB R2012a software was used to investigate betaine release modeling from the designed structures.

Results and discussion

Morphological characteristics

The results of examining the morphological characteristics of pure zein solution (30, 35, and 40%) spun with a voltage of 25 kV and the distance between the tip of the needle and the collector of 250 mm are shown in Fig. 1A-C. Also, the FESEM image of the hybrid electrospun mat of *P. ovata* seed gum/PVA containing 10% w/w betaine extracted from molasses is shown in Fig. 1-D. Based on the results of the FESEM images obtained from electrospinning different concentrations of zein solution, it was found that by increasing the concentration of zein solution from 30 to 35% w/v, the electrospun zein fibers are uniform with a smooth surface (Fig. 1-A and Fig. 1-B). However, increasing the concentration of zein solution from 35 to 40% in 70% ethanol solution significantly ($p < 0.05$) leads to the production of coarse, nonuniform, and wrinkled electrospun fibers (Fig. 1-C). Based on this, as shown in Fig. 1, the electrospun fibers obtained from a 35% w/v concentration of pure zein solution have a smooth, uniform surface without wrinkles and defects (Fig. 1-B). This behavior change is probably related to the concentration and viscosity of the zein solution. Therefore, probably, in concentrations lower or higher than 35%, the resulting solution does not have the appropriate viscosity for spinning in an electric field. When a solution with a suitable viscosity is placed in a high-voltage electric field, it provides enough time to be stretched in the electric field [27, 28]. By providing sufficient time for spinning polymers/biopolymers (with optimal viscosity) in the electric field, the polymer strands are stretched well in the electric field, and this is accompanied by the production of uniform fibers with a smooth and uniform surface. The presence of fibers with a smooth and uniform surface is necessary to create a uniform and timely release profile [29, 30]. These findings were consistent with the results of other researchers. Based on the study of Heydari-Majd et al. (2019), it was found that the suitable concentration for spinning the zein polymer solution is 35%, which leads to the production of uniform electrospun fibers with a smooth surface [11].

Figure 1. FESEM images of zein electrospun fibers with different concentrations A (30%), B (35%), and C (40%), and electrospun mat of *P. ovata* seed gum/PVA containing 10% betaine extracted from molasses (D)

Contact angle

To determine the surface hydrophilicity/hydrophobicity of the films, the contact angle method of the water droplet with the film surface is used [31]. The results of determining the contact angle of the water droplet with the surface of electrospun mats of single-layer electrospun mats of *P. ovata* seed gum loaded with betaine extracted from beet molasses as well as sandwich mats based on zein- *P. ovata* seed gum loaded with betaine are shown in Fig. 2. Based on the obtained results, it was found that the contact

angle of the water drops with the surface of each mat is significantly ($p < 0.05$) related to the type of film. Therefore, as shown in Fig. 2-A, a drop of water forms an angle of 53.5° in contact with the electrospun mats of *P. ovata* seed gum containing 10% w/w betaine. However, by sandwiching these electrospun mats containing betaine using zein electrospun fibers, the contact angle of the water drop with the mat's surface increased significantly (92.45°) (Fig. 2-B). Due to the hydrophobic nature of zein, it is expected that the use of these compounds as a sandwich layer on the electrospun mats of betaine-containing *P. ovata* seed gum will lead to an increase in the surface hydrophobicity of these films and, as a result, an increase in the contact angle of the water droplet with the surface of the films [32]. These findings were consistent with the results of other researchers regarding the contact angle of the water droplet with the surface of the electrospun films. Deng et al. (2018) studied the contact angle of water droplets with the surface of electrospun zein films. Their results showed that the electrospun zein film had a contact angle of 91.5° , which indicates the hydrophobic nature of this biopolymer [33].

Figure 2. Contact angle of the water droplet with the surface of electrospun mats of *P. ovata* seed gum/PVA containing 10% betaine extracted from molasses, (B) sandwich structure

FTIR analysis

The results of FTIR analysis of betaine extracted from sugar beet molasses, electrospun nanofibers of *P. ovata* seed gum containing 10% betaine, electrospun zein fibers, and the sandwich structure consisting of electrospun zein fibers-electrospun fibers of *P. ovata* seed gum containing 10% betaine-electrospun zein fibers is shown in Fig. 3. Betaine extracted from sugar beet molasses has a sharp and broad peak in the region of 3194 cm^{-1} (-OH). Also, in betaine extracted from molasses, there were significant peaks in the areas of 2241 , 2074 , and 1951 cm^{-1} , which are related to C-H bonds in -CH₃ (methyl) or -CH₂ (methylene) groups (Fig. 3). In addition, the stretching vibrations of 1571 , 1477 , and 982 cm^{-1} in the FTIR spectrum of betaine extracted from molasses are caused by N-H, C-N, and C-O bonds, respectively [34]. The pure electrospun PVA/*P. ovata* seed gum hybrid mat also showed peaks in the regions of 3423 , 2850 , 2250 , 1700 , 1400 , 1100 , and 900 cm^{-1} , which are related to O-H, C-H, C=O asymmetric groups, C=O, C-N, C-O symmetric groups, and C-C stretching vibrations, respectively [35] (Fig. 3). The FTIR spectrum of electrospun zein fibers has characteristic peaks at 3283 , 1648 , 1535 , and 1240 cm^{-1} , which correspond to amide-A, amide-I, amide-II, and amide-III, respectively [36] (Fig. 3). FTIR analysis of the sandwich structure consisting of electrospun zein fibers-electrospun fibers of *P. ovata* seed gum containing 10% betaine-electrospun zein fibers shows the index peaks related to pure betaine, electrospun fibers of *P. ovata* seed gum/PVA and pure zein. FTIR peaks of betaine were observed in the region of $2241 - 1950$, 1501 , 1407 , and 1019 cm^{-1} in the structure of sandwich mats (Fig. 3). Also, pure zein peaks were observed in the region of 3300 , 1600 , 1500 , and 1200 cm^{-1} in the FTIR spectrum of the sandwich structure (Fig. 3). These findings confirm the successful encapsulation of betaine in the fiber structure. The findings of this study are consistent with the results obtained by other researchers in terms of FTIR analysis. Based on the study of Charpashlo et al. (2021) on the encapsulation of lycopene in the structure of electrospun gelatin fibers and sandwiching it with electrospun layers of zein, it was determined that the FTIR spectrum obtained from this analysis confirmed the successful encapsulation of lycopene in these

structures. Also, based on the FTIR spectrum obtained from this research, it was determined that the characteristic spectra of lycopene, gelatin, and zein appeared in the sandwich structure, indicating the success of the encapsulation and sandwiching process [37].

Figure 3. FTIR spectra of samples of (A) electrospun mats of *P. ovata* seed gum/PVA containing 10% betaine extracted from molasses, (B) sandwich structure, (C) betaine extracted from molasses, and (D) electrospun mats of zein

DSC analysis

The results of thermal analysis of betaine extracted from sugar beet molasses, electrospun nanofibers of *P. ovata* seed gum containing 10% betaine, and sandwich structure consisting of electrospun zein fibers-electrospun fibers of electrospun containing 10% betaine-electrospun zein fibers are shown in Fig. 4. As shown in Fig. 4, the thermogram spectrum of betaine extracted from sugar beet molasses has two endothermic peaks at around 90°C (with a center of 94°C) and around 290°C (with a center of 293°C), which are probably due to the water evaporation and the destruction temperature of betaine, respectively [38].

Also, the thermogram spectrum of the electrospun nanofibers of *P. ovata* seed gum loaded with 10% betaine extracted from sugar beet molasses has three different endothermic peaks in the region of 59.5°C, 166.5°C, and 302°C (Fig. 4). According to the reported studies, the first peak is probably related to the loss of moisture and the loss of hydroxyl groups, the second peak is probably related to the glass transition temperature (T_g) of the *P. ovata* seed gum/PVA hybrid mats, and finally, the third peak is probably due to the temperature of the overall destruction of this structure [39].

By producing a sandwich structure and using layers of electrospun fibers on both sides of the electrospun fibers of *P. ovata* seed gum loaded with betaine extracted from sugar beet molasses, the position of the peaks related to betaine-loaded fibers changed (Fig. 4). Based on this, as can be seen, the three-layer sandwich structure has an endothermic peak of about 95°C. Also, with the creation of a sandwich structure and the production of electrospun fibers, the second peak of betaine (in the region of 166.5°C) completely disappeared. The disappearance of the second peak related to betaine in the DSC thermogram structure indicates the successful encapsulation of betaine in the designed structures. In addition to these, the third peak of the electrospun fibers of *P. ovata* seed gum in the area of 302°C was moved to a higher temperature of 320°C by producing a sandwich structure (Fig. 4). In addition to confirming the successful encapsulation, this also indicates an increase in the thermal stability of betaine [40]. These findings were consistent with the results of other researchers in terms of thermal properties. Aydogdu et al. (2019) studied the thermal stability of gallic acid loaded in the structure of electrospun lentil flour/polyethylene oxide nanofibers using DSC analysis. Based on the results obtained by these researchers, it was found that the peak related to the thermal degradation of pure gallic acid disappeared in the designed nanofiber structure. Based on the statements of these researchers, it was found that these results indicate the successful encapsulation of gallic acid in the designed structures and increase its thermal stability [41].

Figure 4. DSC thermograms of (A) molasses-extracted betaine, (B) electrospun *P. ovata* seed gum/PVA containing molasses-extracted betaine, and (C) sandwich structure.

In vitro release modeling of betaine

The results of betaine release from the structure of electrospun fibers of *P. ovata* seed gum compared to the sandwich structure (zein-*P. ovata* seed gum containing betaine-zein) were evaluated in the saliva simulated media (10 min), stomach (2 h) and intestine (6 h). (Fig. 5, and Fig. 6). Based on the results obtained during this research, it was found that the electrospun fibers of *P. ovata* seed gum containing betaine in the oral phase completely released the betaine loaded in its structure. Therefore, as shown in Fig. 5, the betaine loaded in the electrospun fiber structure of *P. ovata* seed gum was completely released during the first 1 min of the release test. This action is due to the fast-dissolving and super hydrophilicity of the hybrid electrospun mats of *P. ovata* seed gum/PVA. This fast-dissolving property leads to the rapid dissolution of structures in simulated saliva, which is accompanied by the fast release of betaine loaded in these structures [16]. However, the betaine loaded in the sandwich structure for 10 min in the simulated oral phase had a small release (4.32%) (Fig. 6). When the sandwich structure containing betaine was introduced into the simulated stomach phase of the (2 h), the amount of betaine release increased significantly, and after this time, the amount of released betaine reached 14.85% (Fig. 5). Zein, unlike the hybrid mats of *P. ovata* seed gum/PVA, has a high hydrophobicity property which causes the resistance of the sandwich structure to minimize betaine release in the oral phase. Under acidic condition and pepsin enzyme in the gastric phase are effective factors in betaine release. The outer layer of the sandwich structure is made of zein, which is destroyed under the acidic conditions of the stomach, and pepsin enzyme, which is accompanied by the release of betaine in this media. Therefore, the structural strength of the sandwich matrix is weakened in the stomach phase until this matrix reaches the simulated intestinal phase [42–44].

To investigate the release status of betaine from the sandwich structure loaded with betaine extracted from sugar beet molasses in the simulated intestinal phase, the produced sandwich structure was placed in the simulated intestinal media for 6 h, and the results of its release status are shown in Fig. 6. Based on this, as shown in Fig. 6, the amount of betaine released from the sandwich structure during the first 2 h in the simulated intestinal media reached 58.01%, and then during the remaining 4 h, the amount of betaine remaining (41.99%) was completely released. This behavior is probably caused by the high solubility of hydrolyzed zein from the previous phase. It means that under the acidic and enzyme conditions in the simulated stomach media, the zein protein of the outer layer of the sandwich structure is partially hydrolyzed and provides the conditions for the release of loaded betaine in the simulated intestine media [45].

To investigate the dominant mechanism involved in the release of betaine, the single-layer electrospun structure of *P. ovata* seed gum and the three-layer sandwich structure of Higuchi, Rigter-Peppas, Peppas-Sahlin, and Kopcha mathematical models were used, and the best model was selected based on RMSE and coefficient of explanation (R^2). (Table 1 and Table 2). Based on the results of investigating the

betaine release process, it was found that for single-layer samples containing betaine in the oral phase, the Peppas-Sahlin model was the best model to describe betaine release behavior (Table 1). Also, the fitting of betaine release data from the three-layer sandwich structure in all three different phases with different mathematical models showed that the Peppas-Sahlin model is the best model to describe betaine release behavior in all three phases (Table 2). In addition, the dominant mechanism in the release of betaine from the structure of hybrid mats was investigated using Rigter-Peppas ($n < 0.45$), Peppas-Sahlin ($k_1/k_2 > 1$) and Kopcha ($A/B > 1$) equation constants (Table 1 and Table 2). Based on the Kopcha and Peppas-Sahlin equations, if the ratio A/B and k_1/k_2 are greater than 1, the dominant mechanism affecting the release will be Fickian diffusion [24, 25, 46]. The same law of Fick diffusion is considered for systems with cylindrical shapes if the index $n < 0.45$ in the Peppas-Sahlin model [24, 47]; Therefore, the Fickian diffusion mechanism will be dominant for all samples in the simulated gastrointestinal tract media.

Table 1
Betaine release model parameters from *P. ovata* seed gum nanofiber mats in saliva-simulated media.

Model	Parameters	Parameter responses
Higuchi	k	13.14
	R ²	0.904
	RMSE	11.49
Rigter-Peppas	k	5.56
	n	0.434
	R ²	0.954
	RMSE	8.24
Peppas-Sahlin	k ₁	284
	k ₂	247.1
	m	0.090
	R ²	0.980
	RMSE	5.65
Kopcha	A	6.40
	B	1.06
	R ²	0.947
	RMSE	8.87

Table 2
Model parameters of betaine release from the sandwich structure in the simulated media of digestive tract.

Model	Parameters	Simulated saliva	Simulated stomach	Simulated intestine
Higuchi	k	0.205	1.347	4.260
	R ²	0.872	0.979	0.722
	RMSE	0.586	0.585	16.37
Rigter-Peppas	k	0.259	1.231	0.170
	n	0.421	0.325	0.413
	R ²	0.876	0.981	0.958
	RMSE	0.590	0.584	6.557
Peppas-Sahlin	k ₁	0.094	0.758	1045
	k ₂	0.0005	0.008	837.6
	m	0.724	0.708	0.048
	R ²	0.930	0.986	0.988
	RMSE	0.452	0.517	3.534
Kopcha	A	0.259	1.311	0.529
	B	0.0029	0.0069	0.251
	R ²	0.889	0.980	0.960
	RMSE	0.558	0.595	6.409

Figure 5. Cumulative release of betaine from the structure of electrospun *P. ovata* seed gum nanofiber mats in saliva-simulated media

Figure 6. Cumulative release of betaine from the sandwich structure in saliva-simulated media (blue color), stomach-simulated media (green color), and intestinal-simulated media (red color).

Conclusion

In this study, betaine was extracted from sugar beet molasses and loaded into the structure of electrospun PVA/*P. ovata* seed gum fibers. For the sandwich structure, three different concentrations of zein (30, 35, and 40% w/v) were used as outer layers, and the results showed that the concentration of 35% w/v of zein in 70% v/v ethanol leads to the production of smooth and uniform fibers. The contact angle of the water droplet with the surface of the sandwich structure was significantly ($p < 0.05$) higher than the contact angle of the water droplet with the surface of the electrospun mats PVA/*P. ovata* seed gum containing 10% beet molasses betaine. FTIR and DSC analysis of the designed structures showed that the encapsulation process was successful. The use of a sandwich layer compared to the electrospun single-layer structure of PVA/*P. ovata* seed gum containing betaine extracted from molasses led to an increase in the thermal stability of betaine. The electrospun monolayer structure of PVA/*P. ovata* seed gum containing betaine extracted from molasses was wholly dissolved in the simulated saliva phase and released its betaine completely. However, the sandwich structure released only 4.32% of its betaine in the simulated phase of saliva and 14.85% of its loaded betaine in the simulated phase of the stomach and released the rest in the simulated phase of the intestine. Based on the results of *in vitro* release modeling in simulated digestive system media, it was found that for all the structures of the Peppas-Sahlin model, it was the best model to describe the release behavior, and the Fickian diffusion mechanism was the dominant effective mechanism in betaine release.

Declarations

Acknowledgements

The authors acknowledge Islamic Azad University, Science and Research Branch, Tehran, Iran for their technical support.

Conflict of Interests

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.

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

Not applicable.

Contributions

Zahra Abdollahzadeh: Investigation, Writing - original draft, Validation, Formal analysis. **Masoud Honarvar:** Conceptualization, Methodology, Writing - review & editing, Validation, Supervision, Project administration. **Mehrdad Ghavami:** Writing - review & editing, Supervision.

Availability of data and materials

The datasets used or analyzed during the current study is available from the corresponding author on reasonable request.

Funding

The authors did not receive support from any organization for the submitted work.

References

1. De Zwart, F.J., Slow, S., Payne, R.J., Lever, M., George, P.M., Gerrard, J.A., Chambers, S.T.: Glycine betaine and glycine betaine analogues in common foods. *Food Chem.* 83, 197–204 (2003)
2. Ratriyanto, A., Mosenthin, R., Bauer, E., Eklund, M.: Metabolic, osmoregulatory and nutritional functions of betaine in monogastric animals. *Asian-Australasian J. Anim. Sci.* 22, 1461–1476 (2009)
3. Germec, M., Turhan, I.: Effect of pH control and aeration on inulinase production from sugarbeet molasses in a bench-scale bioreactor. *Biomass Convers. Biorefinery.* 1–13 (2021)
4. Puligundla, P., Mok, C.: Valorization of sugar beet pulp through biotechnological approaches: recent developments. *Biotechnol. Lett.* 1–11 (2021)
5. Mohammadzadeh, M., Honarvar, M., Zarei, A.R., Boojar, M.M.A., Bakhoda, H.: A new approach for separation and recovery of betaine from beet molasses based on cloud point extraction technique. *J. Food Sci. Technol.* 55, 1215–1223 (2018)
6. Ghorani, B., Emadzadeh, B., Rezaeinia, H., Russell, S.J.: Improvements in gelatin cold water solubility after electrospinning and associated physicochemical, functional and rheological properties. *Food Hydrocoll.* 104, (2020). <https://doi.org/10.1016/j.foodhyd.2020.105740>
7. Rezaeinia, H., Ghorani, B., Emadzadeh, B., Mohebbi, M.: Prolonged-release of menthol through a superhydrophilic multilayered structure of balangu (*Lallemantia royleana*)-gelatin nanofibers. *Mater. Sci. Eng. C.* 115, 111115 (2020). <https://doi.org/10.1016/j.msec.2020.111115>
8. Rezaeinia, H., Emadzadeh, B., Ghorani, B.: Electrospun balangu (*Lallemantia royleana*) hydrocolloid nanofiber mat as a fast-dissolving carrier for bergamot essential oil. *Food Hydrocoll.* 100, (2020). <https://doi.org/10.1016/j.foodhyd.2019.105312>
9. Coelho, S.C., Estevinho, B.N., Rocha, F.: Recent Advances in Water-Soluble Vitamins Delivery Systems Prepared by Mechanical Processes (Electrospinning and Spray-Drying Techniques) for Food and Nutraceuticals Applications—A Review. *Foods.* 11, 1271 (2022)
10. Shetty, K., Bhandari, A., Yadav, K.S.: Nanoparticles incorporated in nanofibers using electrospinning: A novel nano-in-nano delivery system. *J. Control. Release.* 350, 421–434 (2022)
11. Heydari-Majd, M., Rezaeinia, H., Shadan, M.R.M.R., Ghorani, B., Tucker, N.: Enrichment of zein nanofibre assemblies for therapeutic delivery of Barije (*Ferula gummosa* Boiss) essential oil. *J. Drug Deliv. Sci. Technol.* 54, (2019). <https://doi.org/10.1016/j.jddst.2019.101290>

12. Komijani, M., Mohebbi, M., Ghorani, B.: Assembly of electrospun tri-layered nanofibrous structure of zein/basil seed gum/zein for increasing the bioaccessibility of lycopene. *LWT*. 161, (2022).
<https://doi.org/10.1016/j.lwt.2022.113328>
13. Kazemianrad, F., Koocheki, A., Ghorani, B.: Encapsulation of caffeine in sandwich structured *Alyssum homolocarpum* seed gum/PVA/gelatin nanofibers using electrospinning technique. *Food Hydrocoll.* 140, 108604 (2023)
14. Heydari-Majd, M., Shadan, M.R., Rezaeinia, H., Ghorani, B., Bameri, F., Sarabandi, K., Khoshabi, F.: Electrospun plant protein-based nanofibers loaded with sakacin as a promising bacteriocin source for active packaging against *Listeria monocytogenes* in quail breast. *Int. J. Food Microbiol.* 110143 (2023)
15. Rezaeinia, H., Ghorani, B., Emadzadeh, B., Tucker, N.: Electrohydrodynamic atomization of Balangu (*Lallemantia royleana*) seed gum for the fast-release of *Mentha longifolia* L. essential oil: Characterization of nano-capsules and modeling the kinetics of release. *Food Hydrocoll.* 93, (2019).
<https://doi.org/10.1016/j.foodhyd.2019.02.018>
16. Abdollahzadeh, Z., Honarvar, M., Ghavami, M.: Modeling the Release of Betaine Extracted from Sugar Beet Molasses in the Structure of Fast-Dissolving Electrospun Fibers of *Plantago ovata* Seed Gum. *Food Biophys.* 17, 448–459 (2022)
17. Yu, L., Yakubov, G.E., Zeng, W., Xing, X., Stenson, J., Bulone, V., Stokes, J.R.: Multi-layer mucilage of *Plantago ovata* seeds: Rheological differences arise from variations in arabinoxylan side chains. *Carbohydr. Polym.* 165, 132–141 (2017)
18. Deng, L., Zhang, X., Li, Y., Que, F., Kang, X., Liu, Y., Feng, F., Zhang, H.: Characterization of gelatin/zein nanofibers by hybrid electrospinning. *Food Hydrocoll.* 75, (2018).
<https://doi.org/10.1016/j.foodhyd.2017.09.011>
19. Tayebi-Moghaddam, S., Khatibi, R., Taklavi, S., Hosseini-Isfahani, M., Rezaeinia, H.: Sustained-release modeling of clove essential oil in brine to improve the shelf life of Iranian white cheese by bioactive electrospun zein. *Int. J. Food Microbiol.* 355, 109337 (2021).
<https://doi.org/10.1016/j.ijfoodmicro.2021.109337>
20. Zarekhalili, Z., Bahrami, S.H., Ranjbar-Mohammadi, M., Milan, P.B.: Fabrication and characterization of PVA/Gum tragacanth/PCL hybrid nanofibrous scaffolds for skin substitutes. *Int. J. Biol. Macromol.* 94, 679–690 (2017)
21. Sarkar, A., Goh, K.K.T., Singh, R.P., Singh, H.: Behaviour of an oil-in-water emulsion stabilized by β -lactoglobulin in an in vitro gastric model. *Food Hydrocoll.* 23, 1563–1569 (2009)
22. Higuchi, T.: Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *J. Pharm. Sci.* 52, 1145–1149 (1963)
23. Ritger, P.L., Peppas, N.A.: A simple equation for description of solute release I. Fickian and non-fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. *J. Control. release.* 5, 23–36 (1987)

24. Peppas, N.A., Sahlin, J.J.: A simple equation for the description of solute release. III. Coupling of diffusion and relaxation. *Int. J. Pharm.* 57, 169–172 (1989)
25. Kopcha, M., Tojo, K.J., Lordi, N.G.: Evaluation of methodology for assessing release characteristics of thermosoftening vehicles. *J. Pharm. Pharmacol.* 42, 745–751 (1990)
26. Alinaqi, Z., Khezri, A., Rezaeinia, H.: Sustained release modeling of clove essential oil from the structure of starch-based bio-nanocomposite film reinforced by electrosprayed zein nanoparticles. *Int. J. Biol. Macromol.* 173, 193–202 (2021). <https://doi.org/10.1016/j.ijbiomac.2021.01.118>
27. Shenoy, S.L., Bates, W.D., Frisch, H.L., Wnek, G.E.: Role of chain entanglements on fiber formation during electrospinning of polymer solutions: good solvent, non-specific polymer–polymer interaction limit. *Polymer (Guildf)*. 46, 3372–3384 (2005)
28. Miri, M.A., Movaffagh, J., Najafi, M.B.H., Najafi, M.N., Ghorani, B., Koocheki, A.: Optimization of electrospinning process of zein using central composite design. *Fibers Polym.* 17, 769–777 (2016)
29. Yan, S., Xiaoqiang, L., Shuiping, L., Xiumei, M., Ramakrishna, S.: Controlled release of dual drugs from emulsion electrospun nanofibrous mats. *Colloids Surfaces B Biointerfaces*. 73, 376–381 (2009)
30. Wongkanya, R., Chuysinuan, P., Pengsuk, C., Techasakul, S., Lirdprapamongkol, K., Svasti, J., Nooeaid, P.: Electrospinning of alginate/soy protein isolated nanofibers and their release characteristics for biomedical applications. *J. Sci. Adv. Mater. Devices*. 2, 309–316 (2017)
31. Ralbag, N., Ruiz-Cabello, F.J.M., Valverde, M.Á.R., Gutkin, V., Sfez, R., Avnir, D.: Continuous thermal control of hydrophilicity/hydrophobicity changes of hybrid films and of their directionality: Kinetics and substrate effects. *J. Colloid Interface Sci.* 505, 692–702 (2017)
32. Ghanbarzadeh, B., Musavi, M., Oromiehie, A.R., Rezayi, K., Rad, E.R., Milani, J.: Effect of plasticizing sugars on water vapor permeability, surface energy and microstructure properties of zein films. *LWT-Food Sci. Technol.* 40, 1191–1197 (2007)
33. Deng, L., Kang, X., Liu, Y., Feng, F., Zhang, H.: Characterization of gelatin/zein films fabricated by electrospinning vs solvent casting. *Food Hydrocoll.* 74, 324–332 (2018)
34. Hu, N., Zhang, K., Li, Y., Hou, T., Zhang, Z., Li, H.: Glycine betaine enhanced foam separation for recovering and enriching protein from the crude extract of perilla seed meal. *Sep. Purif. Technol.* 118712 (2021)
35. Wadhera, P., Jindal, R., Dogra, R.: Synthesis of semi interpenetrating network hydrogel [(GrA-Psy)-cl-Poly (AA)] and its application for efficient removal of malachite green from aqueous solution. *Polym. Eng. Sci.* 59, 1416–1427 (2019)
36. Hosseini, F., Miri, M.A., Najafi, M., Soleimanifard, S., Aran, M.: Encapsulation of rosemary essential oil in zein by electrospinning technique. *J. Food Sci.* 86, 4070–4086 (2021)
37. Charpashlo, E., Ghorani, B., Mohebbi, M.: Multilayered electrospinning strategy for increasing the bioaccessibility of lycopene in gelatin-based sub-micron fiber structures. *Food Hydrocoll.* 113, (2021). <https://doi.org/10.1016/j.foodhyd.2020.106411>
38. Neo, Y.P., Ray, S., Jin, J., Gizdavic-Nikolaidis, M., Nieuwoudt, M.K., Liu, D., Quek, S.Y.: Encapsulation of food grade antioxidant in natural biopolymer by electrospinning technique: A physicochemical study

- based on zein–gallic acid system. *Food Chem.* 136, 1013–1021 (2013)
39. Liang, J., Chen, H., Guo, Z., Dijkstra, P., Grijpma, D., Poot, A.: Tough fibrous mats prepared by electrospinning mixtures of methacrylated poly (trimethylene carbonate) and methacrylated gelatin. *Eur. Polym. J.* 152, 110471 (2021)
 40. Schneider, R., Mercante, L.A., Andre, R.S., Brandão, H. de M., Mattoso, L.H.C., Correa, D.S.: Biocompatible electrospun nanofibers containing cloxacillin: Antibacterial activity and effect of pH on the release profile. *React. Funct. Polym.* 132, 26–35 (2018)
 41. Aydogdu, A., Sumnu, G., Sahin, S.: Fabrication of gallic acid loaded Hydroxypropyl methylcellulose nanofibers by electrospinning technique as active packaging material. *Carbohydr. Polym.* 208, 241–250 (2019)
 42. Zheng, X., Wang, J., Liu, X., Sun, Y., Zheng, Y., Wang, X., Liu, Y.: Effect of hydrolysis time on the physicochemical and functional properties of corn glutelin by Protamex hydrolysis. *Food Chem.* 172, 407–415 (2015)
 43. Li, Y., Liu, H., Liu, Q., Kong, B., Diao, X.: Effects of zein hydrolysates coupled with sage (*salvia officinalis*) extract on the emulsifying and oxidative stability of myofibrillar protein prepared oil-in-water emulsions. *Food Hydrocoll.* 87, 149–157 (2019)
 44. Jiao, Y., Han, H., Chang, Y., Li, D., Riaz, A.: Improvement in entrapment efficiency and in vitro digestion stability of lutein by zein nanocarriers with pepsin hydrolysis. *J. Food Qual.* 2020, 1–9 (2020)
 45. Zayas, J.F., Zayas, J.F.: Solubility of proteins. *Funct. proteins food.* 6–75 (1997)
 46. Fahami, A., Fathi, M.: Development of cress seed mucilage/PVA nanofibers as a novel carrier for vitamin A delivery. *Food Hydrocoll.* 81, 31–38 (2018)
 47. Costa, P., Lobo, J.M.S.: Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Sci.* 13, 123–133 (2001)

Figures

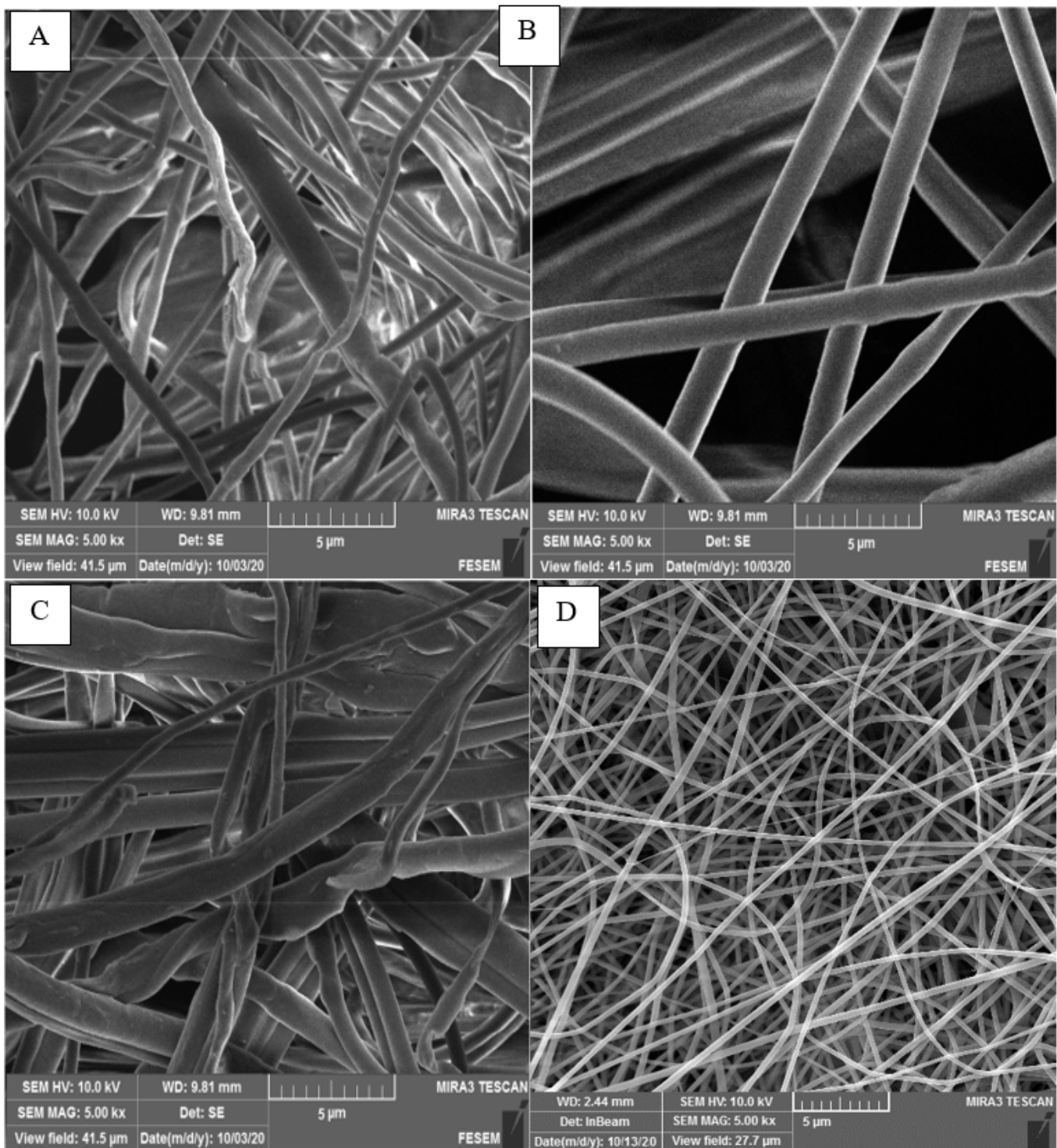


Fig. 1.

Figure 1

FESEM images of zein electrospun fibers with different concentrations A (30%), B (35%), and C (40%), and electrospun mat of *P. ovata* seed gum/PVA containing 10% betaine extracted from molasses (D).

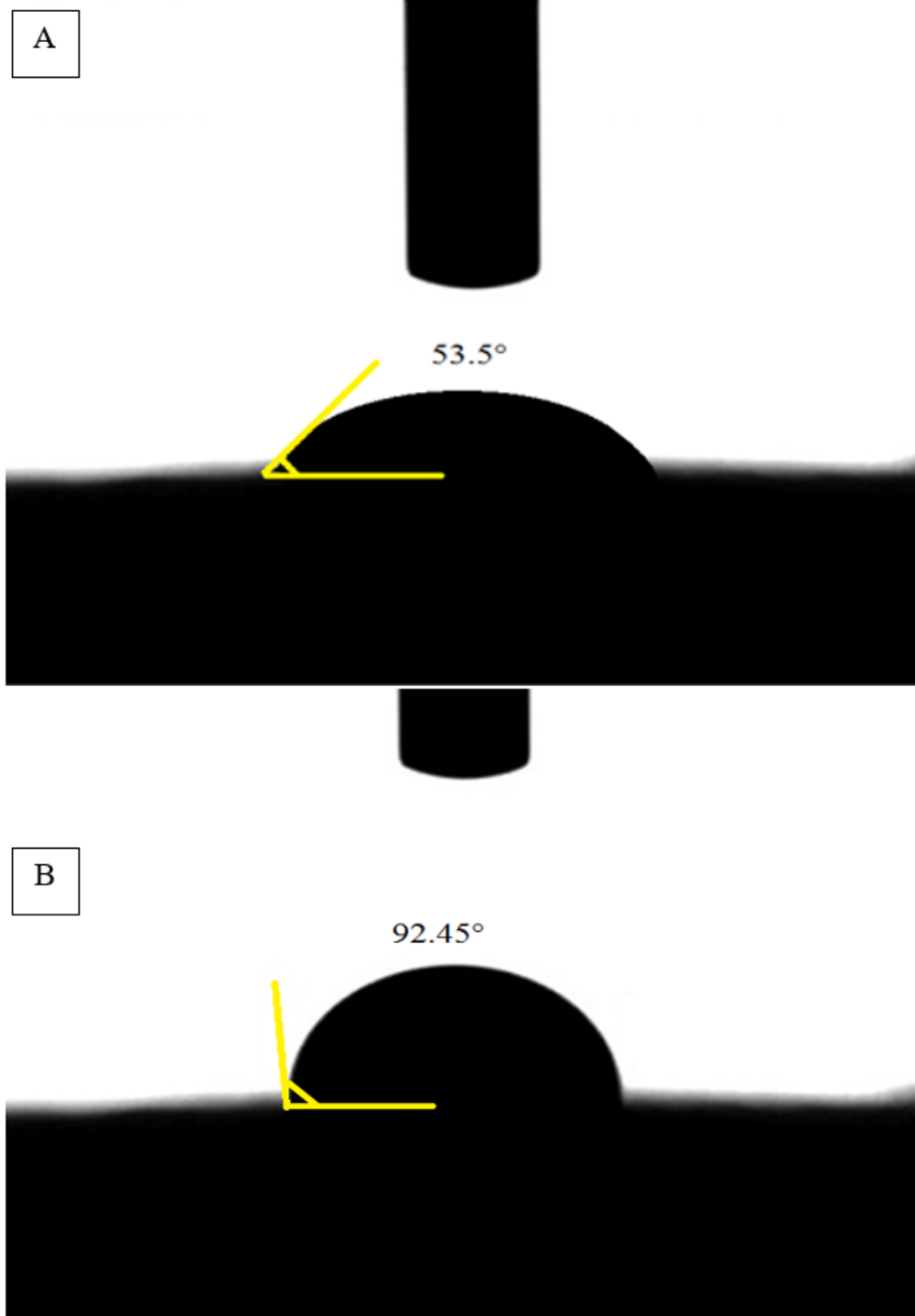


Fig. 2.

Figure 2

Contact angle of the water droplet with the surface of electrospun mats of *P. ovata* seed gum/PVA containing 10% betaine extracted from molasses, (B) sandwich structure.

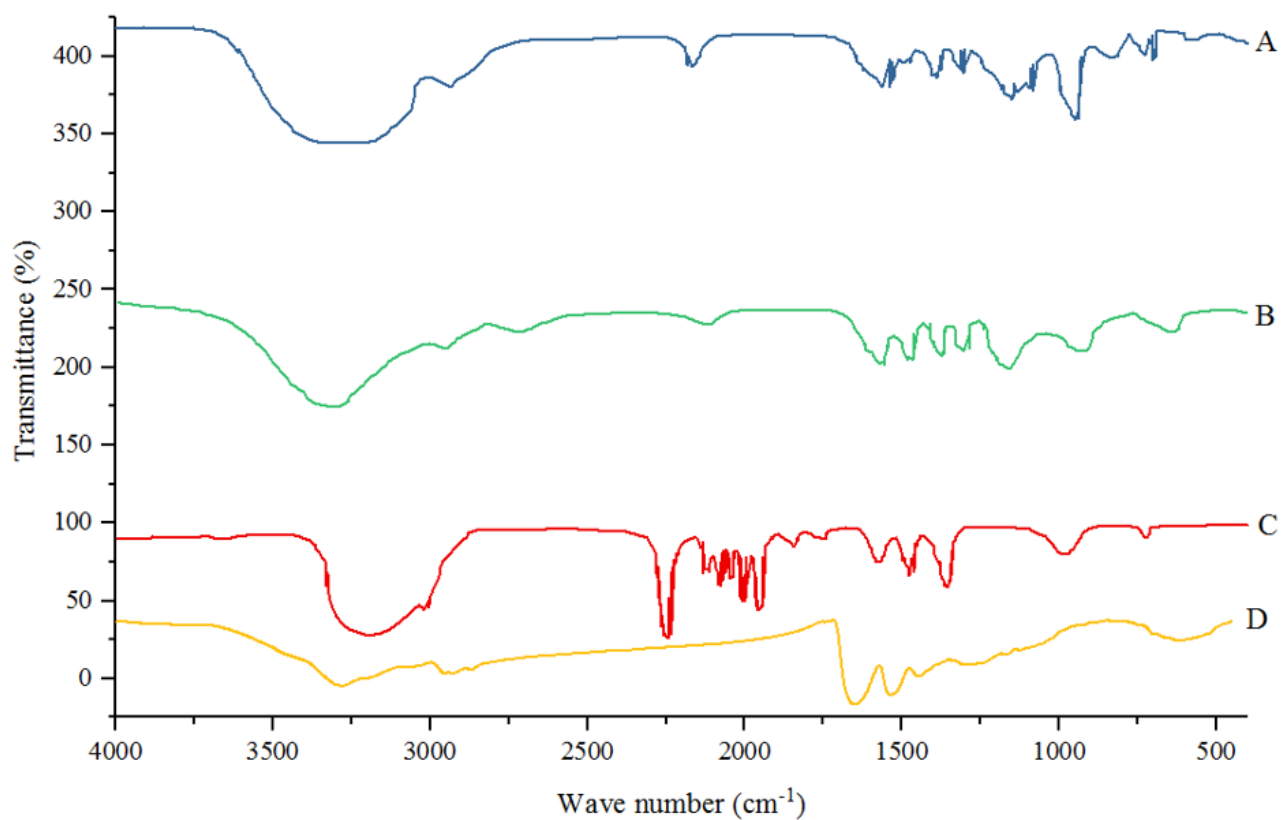


Fig. 3.

Figure 3

FTIR spectra of samples of (A) electrospun mats of *P. ovata* seed gum/PVA containing 10% betaine extracted from molasses, (B) sandwich structure, (C) betaine extracted from molasses, and (D) electrospun mats of zein.

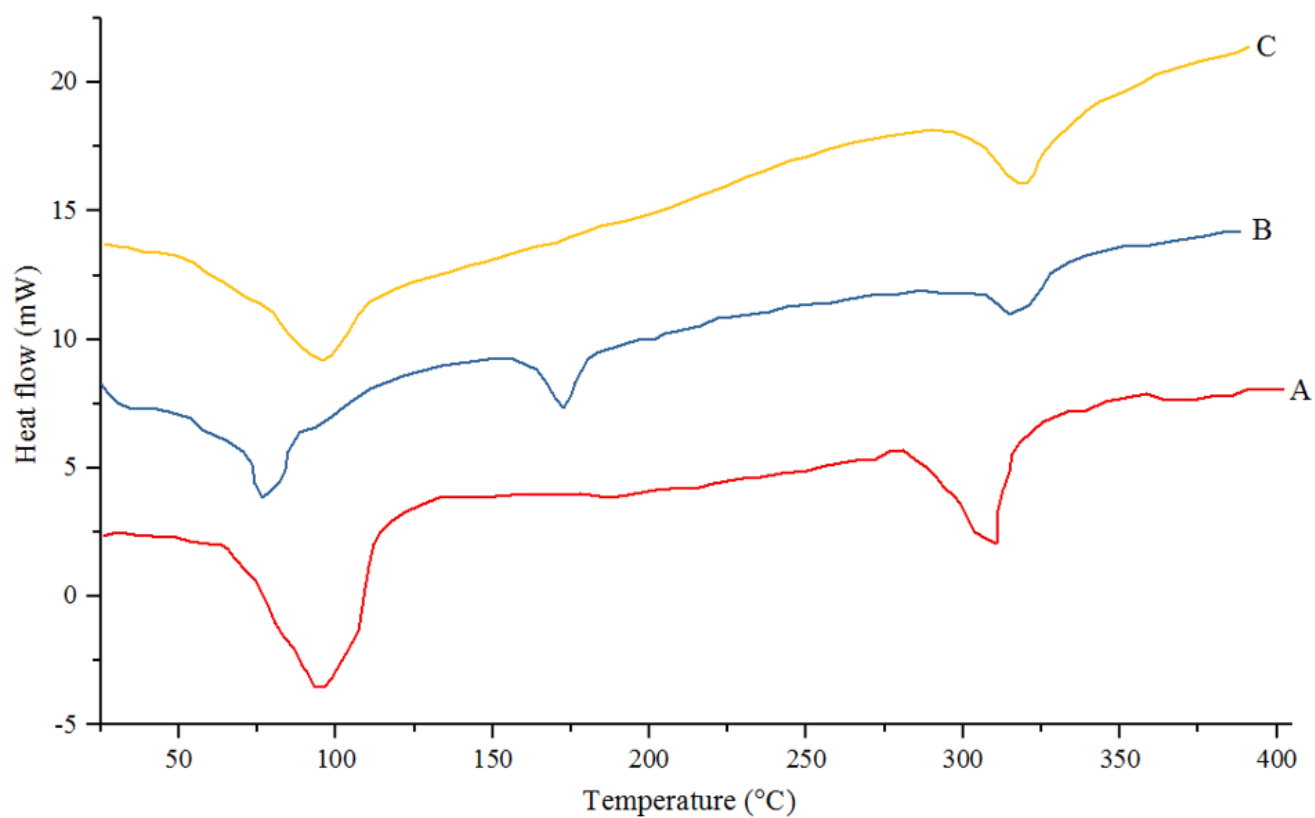


Fig. 4.

Figure 4

DSC thermograms of (A) molasses-extracted betaine, (B) electrospun *P. ovata* seed gum/PVA containing molasses-extracted betaine, and (C) sandwich structure.

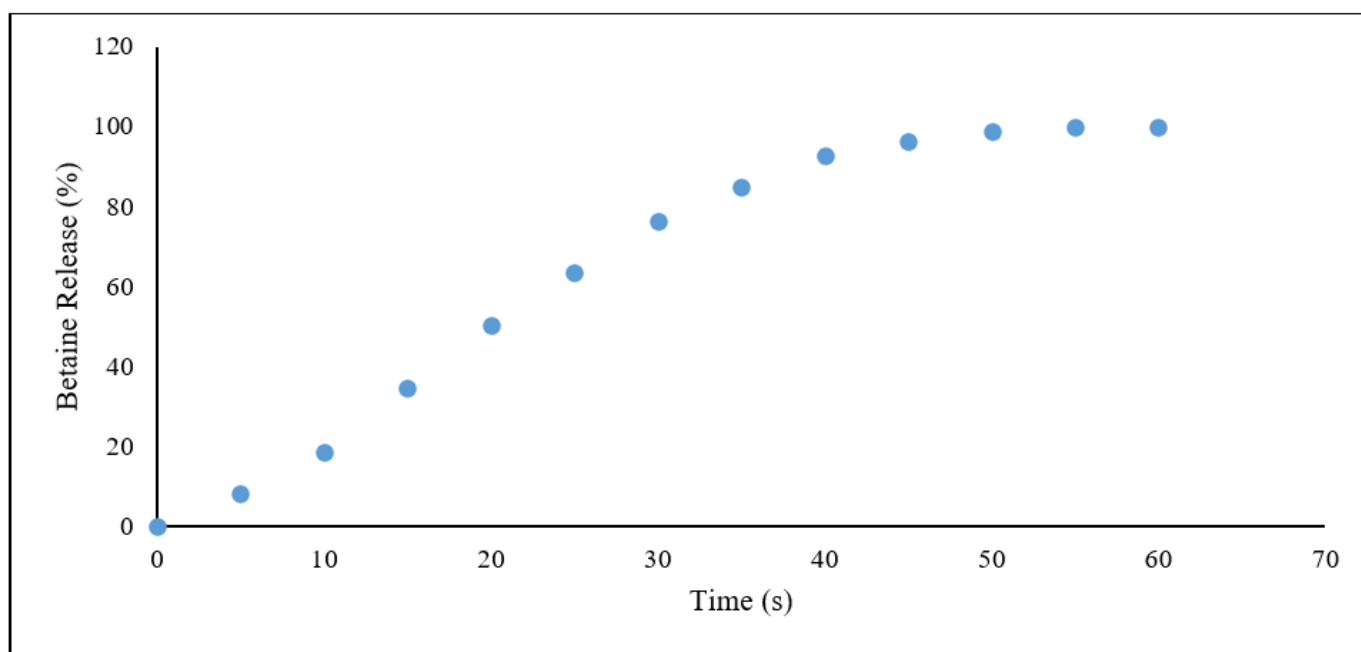


Fig. 5.

Figure 5

Cumulative release of betaine from the structure of electrospun *P. ovata* seed gum nanofiber mats in saliva-simulated media.

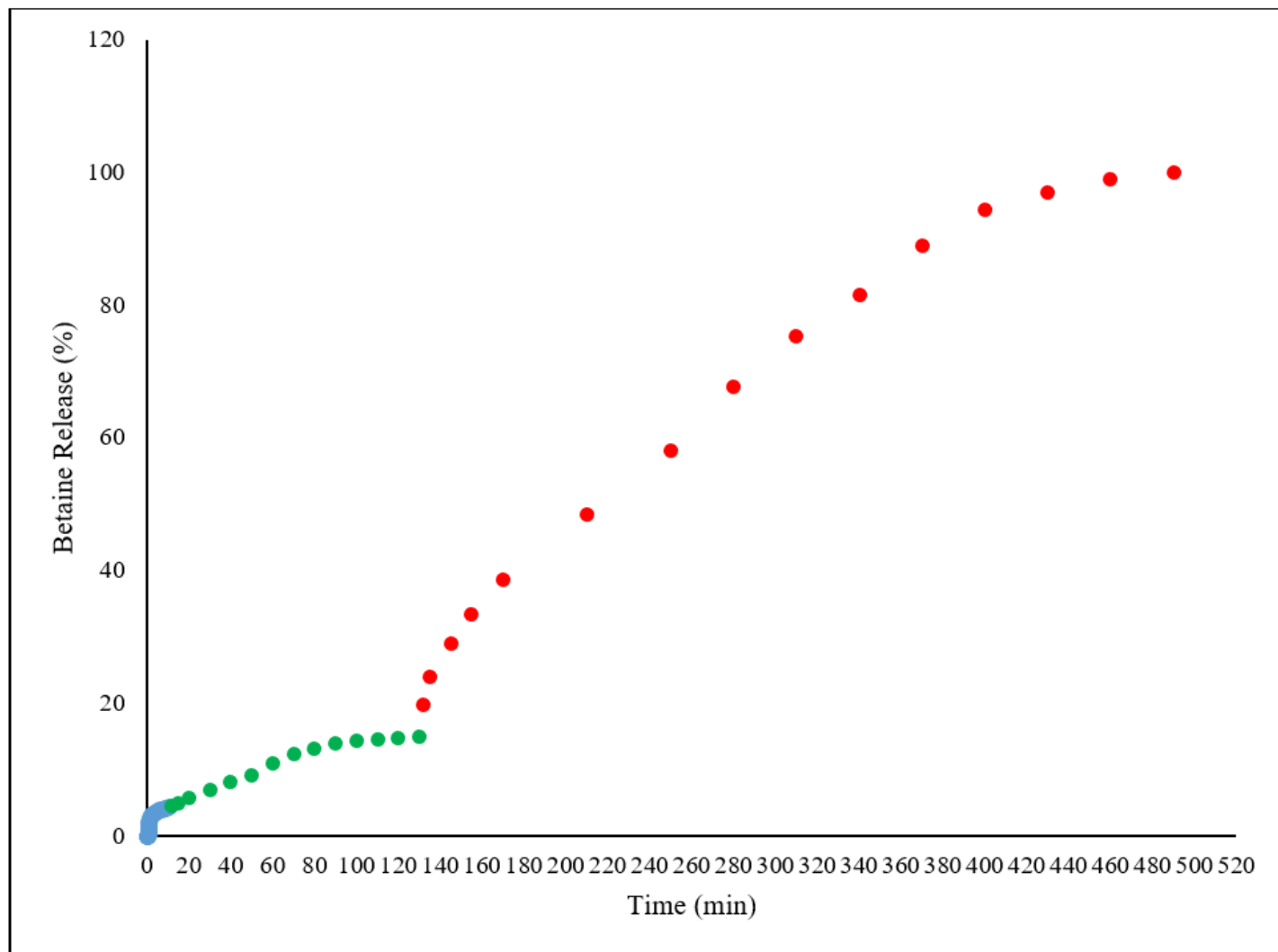


Fig. 6.

Figure 6

Cumulative release of betaine from the sandwich structure in saliva-simulated media (blue color), stomach-simulated media (green color), and intestinal-simulated media (red color).