Food Research 8 (Suppl. 1) : 67 - 77 (2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Effect of cross-linking agents on sodium alginate-based quercetin beads: physicochemical properties and controlled release kinetics

*Prasetyaningrum, A., Rokhati, N., Djaeni, M., Kumoro, A.C., Purwati, D., Hakiim, A., Ashianti, A.D. and Utomo, D.P.

Department of Chemical Engineering, Diponegoro University, Jl. Prof. Sudarto, SH., Tembalang, Semarang 50239, Indonesia

Article history:
Received: 7 March 2023
Received in revised form: 31 October 2023
Accepted: 17 December 2023
Available Online: 3 March 2024

Keywords:
Alginate,
Crosslinking,
Quercetin,
Ionic

DOI:
https://doi.org/10.26656/fr.2017.8(S1).10

Abstract

Alginates have a variety of biological, physical, and chemical properties that can be modified to meet a specific purpose. Ionic crosslinking is an easy method of alginate modification. This study examined the ionic crosslinking agents (Ca^{2+}, Ba^{2+}, Mg^{2+}) on swelling ratio, water solubility, and % sol-gel fraction of modified alginate. The performance of the modified alginate used to encapsulate quercetin, such as % encapsulation efficiency (% EE), % release of quercetin, and quercetin release kinetics were examined. The variables used in this study were the concentration of alginate and ionic crosslinking agents. At 3% alginate concentration and 0.3 M, ionic crosslinking agent concentration positively correlated with swelling ratio and water solubility. In contrast, the ionic crosslinking agent that positively correlates to the % sol-gel fraction is Ca^{2+}. Encapsulation of quercetin using a crosslinking agent with ion Ca^{2+} and 2.5 ppm of quercetin concentration showed the highest % EE of 87%. Modified alginate induced quercetin release from beads at pH 1.4 and 7. The release of quercetin from modified alginate followed the Korsmeyer-Peppas kinetic model (R^2>0.99). The results of the FTIR analysis confirmed that a new peak at 1271 cm^{-1} was C-O-C characteristic of quercetin, indicating quercetin loaded on encapsulated beads. The morphological analysis showed that the surface of modified alginate (Ca-Ag/Ba-Ag/Mg-Ag) was rough and porous. This study established that modified alginate using different ionic crosslinking agents can be a suitable matrix for encapsulating quercetin.

1. Introduction

Quercetin is a polyphenolic flavonoid compound with various uses, including in biotechnology, nutraceuticals, development of new bioactive compounds, complementary drugs, and herbal medicines widely found in vegetables and fruits (Heřmánková *et al*., 2019). Besides that, quercetin is also an antioxidant capable of scavenging free radicals and binding transition metal ions (Tátraaljai *et al*., 2014), anticancer, antibacterial, anti-inflammatory, antiviral, anti-Alzheimer, antifungal and antidiabetic (Jana *et al*., 2018, Chuang *et al*., 2019). Apart from the great benefits of quercetin, quercetin also has several disadvantages, such as poor water solubility, low bioavailability, chemical instability, and short biological half-life, so that it can reduce its efficiency when applied in various applications, including food and medicine (Chavoshpour -Natanzi and Sahihi, 2019). Furthermore, despite five hydroxyl groups, quercetin is known to be lipophilic, giving it low bioavailability (Pool *et al*., 2013). Therefore, it is necessary to develop technology to manipulate materials that can cover the weaknesses of the quercetin but do not reduce the quality of the ingredients and the content contained in them. One of these technological breakthroughs is encapsulation. The selection of an encapsulating agent is crucial for encapsulation efficiency and microcapsule stability. The criteria for selecting wall materials are based on the physical and chemical properties of the material to be encapsulated, such as porosity, solubility, viscosity, mechanical properties, suitability between the two, processing factors and economy (Estevinho *et al*., 2013). Some encapsulants used as coating materials can be natural, semi-synthetic, or synthetic polymers such as alginate, chitosan, gum arabic, ethyl cellulose, gelatin, carrageenan and starch (Ferraz-Carvalho *et al*., 2016). Of the various encapsulants, alginate is the best because of its biodegradability, biocompatibility, low toxicity, relatively low cost, light gelation, and safety in food processes (Sreekumar and Bindhu, 2019). While other

*Corresponding author.
Email: *ajiprasetyaningrum@gmail.com*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

encapsulants still have drawbacks, such as chitosan, which has lower mechanical properties with a high price and unobtrusive performance. Gum arabic has high price and availability problems; ethyl cellulose is insoluble in the gastrointestinal system. And gelatin has low mechanical resistance and is highly soluble in water. Carrageenan dissolves only at high temperatures, where it can damage quercetin in high temperatures. The starch has viscosity too high for most encapsulation processes, and cellulose can slow down drug absorption (Gharsallaoui *et al*., 2007; Estevinho *et al*., 2013; Zeng *et al.,* 2018). The weakness of alginate is its instability when in the form of a gel, so the selection of methods for modifying alginate as an encapsulation matrix needs to be considered. Alginates can react with multivalent cations. Several encapsulation methods can be used: spray drying, emulsification, coacervation, ionic crosslink, freeze drying, and electrospray. Spray drying has high efficiency and can be carried out on a large scale. Still, the encapsulating agent is limited and can eliminate the bioactivity of compounds during the heating process. Only high thermal stability compounds can withstand the process. The emulsification method has high efficiency and reproducibility. Still, the active compound may be lost during solvent evaporation and is unsuitable for high volatile compounds, so it is inappropriate to use (Ezhilarasi *et al*., 2014). The conservation method can be used to encapsulate unstable compounds, and the encapsulation efficiency is high, but the production cost is high (Nesterenko *et a*l., 2013). While freeze drying can be carried out at low temperatures and pressure, it is suitable for compounds that are sensitive to heat but have disadvantages such as high energy requirements, long processing time, and produce unstructured and exposed particles (Raja *et al*., 2019). The electrospray method is suitable for temperature-sensitive bioactive compounds, but only small amounts of particles are produced (Shang *et al*., 2017). Meanwhile, the ionic crosslink method is a method with a straightforward approach, simple steps, controlled release, and a more extended encapsulation period. However, only specific compounds can be encapsulated, and a more crosslinkers agent is needed. Alginates have different affinities for different types of divalent ions (Haug and Larsen, 1971). Alginates can crosslink ionically with multivalent cations such as Pb, Cu, Cd, Ba, Sr, Ca, Co, Ni, Zn, Mn and Mg ions. Crosslinking polymeric microparticles are advantageous compared to non-crosslinked microparticles because they can reduce unwanted decomposition of microparticles and slow down the drug release rate (Dhanka *et al*., 2018). Alginate gel formation can occur in the presence of coordination between alginate and the number of binding cations (Hu *et al*., 2021). Among the above cations, the gelation of alginates induced by Ca^{2+}, Ba^{2+} and Mg^{2+} has been well studied and has good crosslinking strength and has been reviewed in terms of gelation mechanism, gel properties, influencing factors, and applications in the food industry (Petrova *et al*., 2019).The novelty of this study is the effect of ionic crosslinking agents (Ca^{2+}, Ba^{2+} and Mg^{2+}) and the study of quercetin release kinetics using the alginate-modified Ca-Ag/Ba-Ag/Mg-Ag matrix. Therefore, this study mainly aimed to increase the psycho-chemical properties of bead gel of alginate modified Ca-Ag/Ba-Ag/Mg-Ag by the ionic crosslinking method as an encapsulation matrix of quercetin. The swelling ratio, % water solubility, and % sol-gel fraction was evaluated in this study to assess the mechanical properties. In addition, the morphology and chemical structure of Ca-Ag/Ba-Ag/Mg -Ag-based bead gel were characterized. The control of quercetin release will also be evaluated through % encapsulation efficiency, % release, release kinetics studies, and mechanism of the release of quercetin encapsulated Ca-Ag/Ba-Ag/Mg-Ag matrix.

2. Materials and methods

2.1 Materials

Sodium alginate (molecular weigh 216.12 g/mol and CAS Number 9005-38-3), 95% quercetin (CAS number 117-39-5 Sigma-Aldrich), calcium chloride ($CaCl_2$ molecular weight 110.99 g/mol and CAS Number 10043 -52-4), $BaCl_2$ (molecular weight 208.25 g/mol and CAS number 10361-37-2), $MgCl_2$ (molecular weight 95.21 g/ mol and CAS number 7786-30-3), sodium hydroxide (NaOH), and hydrochloric acid (HCl) was purchased from Sigma-Aldrich, Germany.

2.2 Preparation of alginate modified (Ca-Ag/Ba-Ag/Mg -Ag) used ionic crosslinking method

Sodium alginate (1%, 2%, 3%, 4% and 5% w/v) was dissolved in 100 ml of distilled water under constant stirring at 70°C until a homogenous solution was achieved. Then, the temperature was reduced to 27°C under constant stirring. After all mixture was homogenous, 250 mL $CaCl_2$, $BaCl_2$, and $MgCl_2$ solutions with various concentration of divalent ion (0.1 M, 0.2 M, 0.3 M, 0.4 M and 0.5 M w/v) were prepared. The crosslinked matrix solution was dripped into divalent ion ($CaCl_2$, $BaCl_2$ and $MgCl_2$) solution using a syringe with an outlet diameter of 0.5 mm. The formed beads were soaked in the divalent ion solution for 1 hr and then diluted by adding 250 mL of distilled water. The beads were then dried and stored at 27°C.

https://doi.org/10.26656/fr.2017.8(S1).10

2.3 Quercetin encapsulation with Ca-Ag/Ba-Ag/Mg-Ag matrix

The preparation of the modified alginate (Ca-Ag/Ba-Ag/Mg-Ag) solution were as follows. Into a modified solution of Ca-Ag/Ba-Ag/Mg-Ag, 2% w/v of quercetin was added and stirred until dissolved. The quercetin-containing matrix solution was then dripped carefully into a 250 mL divalent ion ($CaCl_2$, $BaCl_2$ and $MgCl_2$) solution using a syringe with an outlet diameter of 0.5 mm. Then the beads were soaked in divalent ion ($CaCl_2$, $BaCl_2$ and $MgCl_2$) solution. Quercetin-loaded beads were filtrated from the divalent ion ($CaCl_2$, $BaCl_2$ and $MgCl_2$) solution and then kept in distilled water for 24 hrs for further usage. A schematic illustration of quercetin encapsulation using modified alginate (Ca-Ag/Ba-Ag/Mg-Ag) is presented in Figure 1. The % EE of quercetin was evaluated by analyzing the concentration of quercetin in the beads using UV spectrophotometry (Shimadzu UV-1900i) at 485 nm. The % EE of quercetin was calculated using:

$$\text{Encapsulation Efficiency (\%)} = \frac{M_i - M_f}{M_i} \times 100 \quad (1)$$

Where Mi, the amount of quercetin and Mf, the amount of bioactive quercetin present in the divalent ion solution after encapsulation.

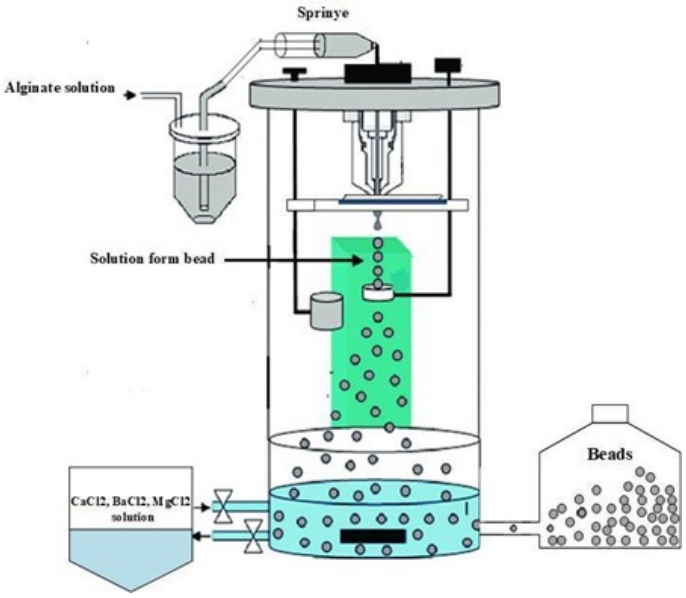

Figure 1. Schematic of the ionic crosslinking process.

2.4 Quercetin release study

A control release study of quercetin was performed using UV-Vis spectrophotometric method. First, 0.1 g of the bead and 20 ml of solution (pH 1.2 and 7.4) were filled into a beaker glass. The solution was stirred for 160 mins, and 2 mL of solution was sampled periodically every 20 mins for free quercetin analysis. Accurately 1 ml of 2% $AlCl_3$ and 1 ml of 1.2 M potassium acetate were added to the sample. The solution was let to stand for 10 mins for color development with continuous stirring. The absorbance of the solution was measured using a UV-vis spectrophotometer at a wavelength of 435 nm. The standard calibration curve was developed for quantification in the UV-Vis spectrophotometer (Shimadzu UV-1900i) analysis. Percent release of quercetin was calculated:

$$\text{\% Release of quercetin} = \frac{C_t}{C_0} \times 100\% \quad (2)$$

Where, C_t, the amount of quercetin release at time (20, 40, 60, 80, 100, 120, 140 and 160 mins); C_0 the total of quercetin in the first solution.

2.5 Quercetin release kinetics study

The mathematical fitting models are applied to confirm and explain the release behavior of quercetin from loaded, modified alginate (Ca-Ag/Ba-Ag/Mg-Ag based bead gels in pH 4.1 and 7.4 following the Zero Order, Higuchi model and Korsmeyer-Peppas model:

$$C_t = C_0 + k_0 t \quad (3)$$

$$\frac{C_t}{C_{total}} = k_H t^{0.5} \quad (4)$$

$$\frac{C_t}{C_{total}} = k_p . t^n \quad (5)$$

Where C_t is the concentration of quercetin released at the time "t" (ppm) and C_0 is the initial concentration of quercetin in the beads (ppm). k_0, k_H, and k_p are the Zero-order, Higuchi, and Korsmeyer-Peppas release constant, respectively. The n is the release exponent to determine the release mechanism. The fitting model of the release kinetics was evaluated using the linearization method.

2.6 Swelling degree analysis

The swelling ratio of modified alginate (Ca-Ag/Ba-Ag/Mg-Ag) bead was calculated by measuring all hydrogels' average percentage of swelling degree. The swelling test was carried out in a solution of pH 1.2 and pH 7.4. The swelling ratio can be calculated using:

$$\text{Swelling ratio} = \frac{W_s - W_d}{W_d} \quad (6)$$

Where W_s is the weight of swollen hydrogel and W_d is the weight of dry gel.

2.7 Solubility analysis

The dried bead gel (0.1 g) was added to water with pH 1.2 and 7.4 and stirred until the bead was dissolved. The solution was centrifuged at 3300 rpm for 15 mins. A total of 10 mL of the supernatant was transferred to a previously weighed petri dish and dried at 105°C to a constant weight. Solubility (g. 100 g 1 water) was calculated as the percentage of dry supernatant with initial weighted powder (1 g) (Mar *et al.*, 2020).

https://doi.org/10.26656/fr.2017.8(S1).10

$$\% \text{ Solubility} = \frac{W_d}{W_s} \times 100 \quad (7)$$

Where W_d is the weight of dry beads, and W_s is the weight of supernatant.

2.8 Sol-gel fraction analysis

The bead gel was dried in an oven at 45°C until it showed a constant weight (W_o). It was then extracted for 4 hrs in distilled water as a solvent. The uncross linked polymer (alginate) will be separated or lost by this extraction from the gel structure. Next, the extracted gel was dried in the oven at 45°C to constant weight (W_1). The gel fraction can be determined using the formula below:

$$\text{Sol fraction } (\%) = \left[\frac{W_o - W_1}{W_0}\right] \times 100\% \quad (8)$$

$$\text{Gel fraction } (\%) = 100 - \text{sol fraction} \quad (9)$$

2.9 Fourier transformed infrared

The modified alginate (Ca-Ag/Ba-Ag/Mg-Ag) and matrix-loaded quercetin were analyzed by FTIR spectral analysis. It was performed using the KBr pellet method on a Perkin Elmer Spotlight 200i spectrophotometer (Perkin Elmer Inc., US) to confirm the information on chemical bonds or functional groups. The spectrum was recorded in 4000-400 cm^{-1} (Karaman, 2019).

2.10 Scanning electron microscopy

The morphological properties of modified alginate Ca-Ag/Ba-Ag/Mg-Ag matrix with encapsulated quercetin were evaluated using SEM (JEOL JSM 6510 La, Tokyo, Japan) to determine the morphological changes caused by the encapsulation process. In addition, the particle size of each sample at a specific magnification of the SEM image was determined using ImageJ image analysis software (Version 1.50i).

3. Results and discussion

3.1 Scanning electron microscopy analysis

Comparing crosslinking agents Ca^{2+}, Ba^{2+} and Mg^{2+} on the modified alginate in beads gel were investigated by scanning electron microscopy (SEM). Micrographs of the alginate modified surface are shown in Figure 2. The surface of beads, in general, are rough surface morphology and visible wrinkles. It can be seen that the addition of divalent ions to alginate can increase the stability of the gel so that it forms smaller surface pores and makes it look more amorphous. Divalent ions Ca, Ba and Mg have different affinities for alginate. The greater the association of the divalent ion for the alginate, the more stable and robust the gel beads will be. In Figure 2A, the surface morphology of the Ca^{2+} ion is rougher than Ba^{2+} and Mg^{2+}.

Meanwhile, adding Mg^{2+} to alginate makes the gel less rigid and looks smoother morphologically. Figure 2B also shows that the addition of Ba^{2+} to the alginate makes the surface of the bead gel morphologically rough, even though it is not rougher than the Ca^{2+} ion. This is thought to be due to the lower solubility of Ba^{2+} than Ca^{2+}, so ion accumulation is possible on the surface during the drying process (Bierhalz *et al*., 2014).

Morphological analysis of quercetin that has been encapsulated using a modified alginate matrix can be seen in Figure 2D. Quercetin encapsulated using Ca-Alginate matrix showed a more-rough and amorphous surface. This indicates that quercetin was successfully trapped in the matrix and adsorbed the bioactive. This is in agreement with the findings by Lacerda *et al*. (2014) that adding rifampicin causes the alginate surface to become rougher.

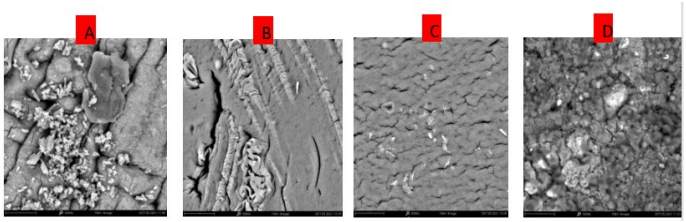

Figure 2. SEM morphological analysis of modified Ca/Ba/Mg/ Alginate encapsulations (A) Ca/Alginate, (B) Ba/Alginate, (C) Mg/Alginate and (D) Ca/Alginate/Quercetin.

3.2 Fourier transformed infrared

The FTIR spectra of modified alginate with divalent ion Ca^{2+}, Ba^{2+} and Mg^{2+} after quercetin was loaded were shown in Figure 3. FTIR analysis was performed on empty and encapsulated beads containing quercetin using crosslinker agents in the form of Ca^{2+}, Ba^{2+} and Mg^{2+}. In the wide strain band at 3500-3100 cm^{-1}, the N-H

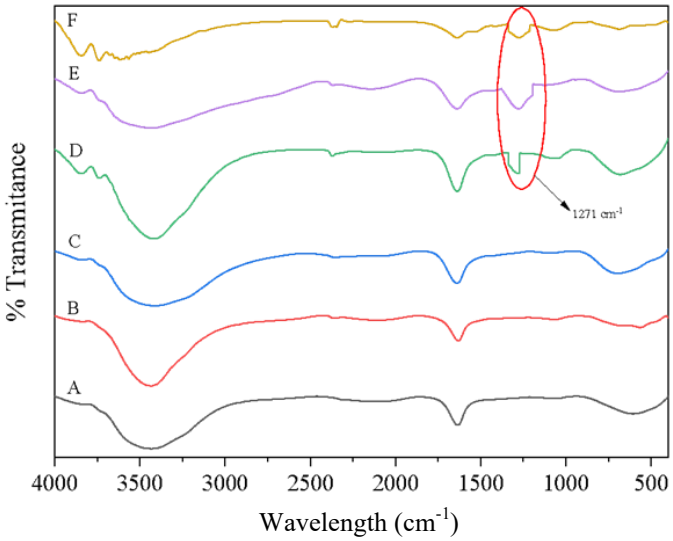

Figure 3. FT-IR Analysis of Modified Ca/Ba/Mg/Alginate: (A) Ca/Alginate (B) Ba/Alginate (C) Mg/Alginate (D) Ca/ Alginate/Quercetin (E) Ba/Alginate/Quercetin (F) Mg/ Alginate/Quercetin.

https://doi.org/10.26656/fr.2017.8(S1).10

stretching vibrations overlap with the OH stretching vibrations. Furthermore, the characteristic spectral band of alginate is seen at 1645 cm^{-1}, indicating the presence of C=O strain vibrations and O-H torsion vibrations of carboxylic acids (Lim and Ahmad, 2017). Although Figures 2A, 2B and 2C show approximately Ca-Alginate, Ba-Alginate and Mg-Alginate, there is no significant difference in the spectra results.

Figure 3 show the FTIR spectra of the encapsulated beads. The results showed several peaks almost having the same value as the presence of N-H, -OH and carboxylic acid groups in some peaks. In this quercetin loading, each new peak was located at 1271 cm^{-1}. This peak can show the presence of C-O-C groups, which indicate quercetin loading in both Ca/Ag/Qe, Ba/Ag/Qe and Mg/Ag/Qe encapsulated beads (Hao et al., 2017). Thus, alginate with modified crosslinker agents Ca, Ba, and Mg successfully encapsulated quercetin in each treatment.

3.3 Swelling behaviour

Swelling behaviour of alginate modified Ca-Ag/Ba-Ag/Mg-Ag beads that occurred due to types in crosslinking agents and pH. The immersion was carried out to determine the effect of swelling for 120 mins. The result of crosslinking agent and media pH on the swelling behaviour of alginate-modified Ca-Ag/Ba-Ag/Mg-Ag beads can be seen in Figure 4.

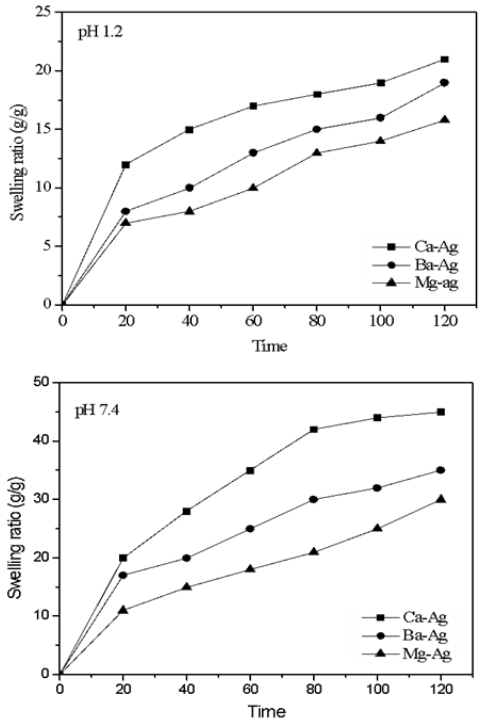

Figure 4. Swelling behaviour of alginate modified Ca-Ag/Ba-Ag/Mg-Ag beads on the different crosslinking agents and pH.

Swelling characteristics are the intrinsic properties of hydrogels. The gel enlarges due to the penetration of water or solvent into the space between the polymer chain network, which in some cases is triggered by pH and ionic strength (Guarrotxena and Quijada-Garrido, 2016). This affects the mass transfer characteristics (diffusion of nutrients and oxygen) and their mechanical properties (Mihaila *et a*l., 2013). For example, the swelling behaviour of alginate modified Ca-Ag/Ba-Ag/Mg-Ag beads is shown in Figure 4, it can be seen that the swelling ratio (g/g) increased significantly with increasing immersion time. This phenomenon is caused by the increasing number of H+ and OH- ions that bind to the hydrophilic group of alginates (COO group - a carboxylic acid that quickly absorbs water) (Barleany *et al*., 2017). So that the polysaccharides will absorb more water and the swelling ratio will increase. Figure 4 shows the maximum swelling ratio that occurred in the crosslinking agent types with divalent Ca^{2+} ions of about 45 g/g and 22 g/g at pH 7.4 and 1.2, approximately. Although the divalent ion Ba^{2+} has the highest affinity for alginate, the selective binding of Ba^{2+} ions to the alginate makes the gel beads too tight and stiff, hindering water absorption on the gel surface. This follows the results of the previous morphological analysis, where the character of Ca^{2+} is rougher than Ba^{2+} and Mg^{2+}, so water adsorption on the gel surface at C^{2+} is better than Ba^{2+} and Mg^{2+} ions.

The media was used to analyze the swelling ratio and % solubility in pH 1.2 and 7.4. In Figure 4, the swelling ratio and % solubility have a good response at pH7.4 by showing a higher value than pH1.2. This phenomenon is because alginate will not dissolve under acidic conditions. After all, it forms an insoluble framework (Sriamorn-sak *et al*., 2007; Chuang *et al*., 2017).

At pH <3.4, the concentration of H+ in high solution reacts with the carboxyl group on alginate to form a non-ionic compound (-COOH) and reduce the number of carboxyl ions in the alginate so water adsorption will decrease and swelling gel will decrease at pH 1.2 (Jao *et al*., 2009).

Meanwhile, at a lower pH concentration of H+ ions (pH>4.4), more carboxyl groups will result in polymer chain expansion and swelling of a hydrophilic matrix (Agüero *et al*., 2017). As a result, the swelling ratio of the modified alginate at pH 7.4 was higher than pH 1.2. Likewise, % solubility is proportional to the swelling ratio. Wardhani *et al*. (2021) also reported that pH 6.8 increased swelling and % solubility value more than pH 1.2.

3.4 Water solubility behavior

The solubility behavior has been used as the main parameter for a bead matrix hydrogel. This study examines the effect of crosslinking agents and pH media

https://doi.org/10.26656/fr.2017.8(S1).10

on solubility behavior. Figure 5 shows the increase in solubility in line with the increase in the immersed time. The longer the bead gel is in contact with water, the longer the water adsorption process to the surface, and stirring during the analysis process also makes the % solubility of the bead gel greater.

The effect of the type of crosslinking agent on the solubility % is also shown in Figure 5. The divalent ions used are Ca^{2+}, Ba^{2+} and Mg^{2+}. Ca^{2+} ions give a greater influence on the solubility of about 25%. Followed by Ba^{2+} and Mg^{2+} which are 20% and 18% at pH 7.4. The crosslinking process involves divalent ions (Ca^{2+}, Ba^{2+} and Mg^{2+}) with the carbonyl group bonding, namely the G block (guluronate) part of the alginate which forms an "egg box" model so that the gelation process (gel form) occurs (Hu *et al*., 2021). Each type of divalent ion between Ca^{2+}, Ba^{2+} and Mg^{2+} has a different selectivity when carrying out the crosslinking process. The Ca^{2+} ion has a low selectivity to alginate in the crosslinking process. Meanwhile, Ba^{2+} and Mg^{2+} are more selective during the crosslinking process (Ardiles *et al*., 2021). This is because Ca^{2+}, Ba^{2+} and Mg^{2+} have different binding modes on alginate. Calcium with carbonyl groups, namely G and MG blocks, barium with G and MM blocks. Between Ca and Ba the main binding mode is with G block, while Mg with carboxylate, namely uronate and the main is MG block so that the gel formed is less stable than Ca^{2+} and Ba^{2+} (Cao *et al*., 2020; Hasan *et al*., 2020). As a result, it affects the psychochemical properties of water solubility of alginate.

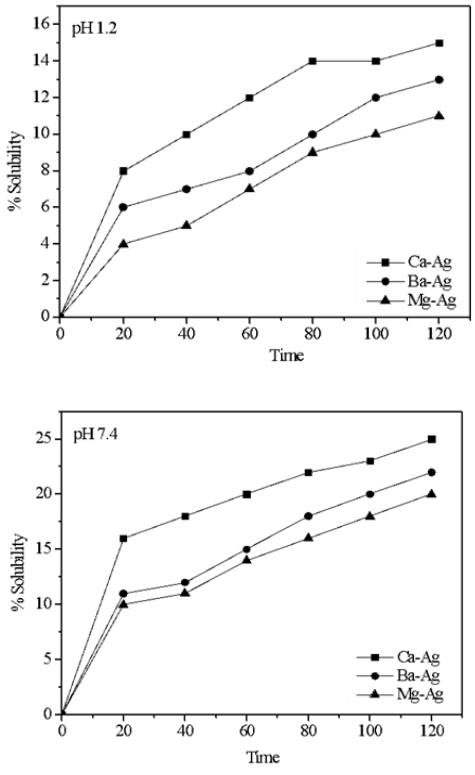

Figure 5. Solubility behavior of alginate modified Ca-Ag/Ba-Ag/Mg-Ag beads on the different crosslinking agents and pH.

3.5 Study of encapsulation efficiency of quercetin with Ca-Ag/Ba-Ag/Mg-Ag matrix

The encapsulation process is expected to protect quercetin from the degradation of antioxidant compounds. In addition, alginates have promising properties related to their sensitivity to pH and ability to target a drug release (Agüero *et al*., 2017). Therefore, they combined quercetin with alginate with the best alginate formulation and crosslinking agent concentration. In this study, the best alginate concentration is 3%, and the crosslinking agent is 0.5 M. In the % efficiency analysis, crosslinking agent and quercetin concentration were used to determine how effectively quercetin encapsulation using the best formulation of alginate and crosslinking agent likes in Figure 6.

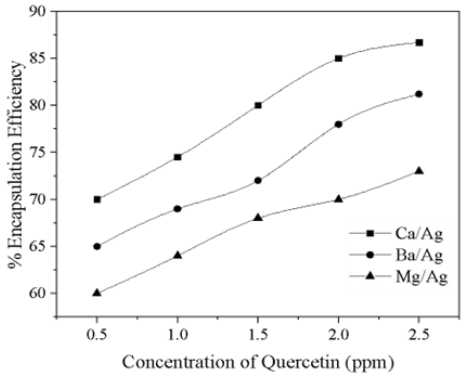

Figure 6. Effect of crosslinking agent and quercetin concentration on the percentage of encapsulation efficiency.

Figure 6 shows the type of crosslinking affects the results of the % efficiency. Covalent ion crosslinking type Ca^{2+} has the highest % encapsulation efficiency compared to Ba^{2+} and Mg^{2+}. At Ca^{2+}, it can achieve 87% efficiency, while Ba^{2+} and Mg^{2+} are 80% and 72%. In this case, % encapsulation efficiency is influenced by the ability of alginate to form a gel (Draget *et al.,* 2006). The quercetin encapsulation process in this study was carried out by forming beads in crosslinking solution. The formation of beads occurs due to the crosslinking process between divalent ions $Ca^{2+,}$ Ba^{2+}, and Mg^{2+} with alginate, where divalent ions replace Na+ ions in sodium alginate (Ardiles *et al*., 2021).

In Figure 6, it can be seen that the Na^{+} is bound to the GG (carboxyl) block group. The binding of Ca^{2+} and Ba^{2+} ions on the GG block and Mg^{2+} in the uronate group on the MG block forms an "egg box" (Hu *et al*., 2021). This can affect the gel's stability and alginate's ability to encapsulate quercetin. Ca^{2+} with not very selective and has more stable reactive properties and high % encapsulation efficiency than others due to good gel formation. Meanwhile, the unstable Mg^{2+} result in a % encapsulation efficiency is low (Topuz *et al.*, 2012).

https://doi.org/10.26656/fr.2017.8(S1).10

This study also analyzed the effect of encapsulated active ingredient (quercetin) concentration. Variable concentrations of quercetin used were 0.5,1, 1.5, 2 and 2.5 ppm. Figure 7 shows the higher quercetin concentration increases % encapsulation efficiency. The encapsulation process with the ion crosslinking method was done by dissolving quercetin with ethanol and mixing it with an alginate solution. Then, the solution dropped into crosslinking agent solution (Ca^{2+}, Ba^{2+} and Mg^{2+}). This encapsulation process is called the exogenous method, which occurs simultaneously and quickly, so a concentration gradient of crosslinking agents and active compounds on the surface of alginate gel (Ramdhan *et al*., 2019). The gradient of quercetin concentration on the gel surface is influenced by the concentration of quercetin in the alginate solution. The higher concentration of quercetin affects the quercetin component encapsulated in alginate. Guo *et al*. (2018) reported that variations of encapsulated compound concentration would increase encapsulation efficiency even though they are insignificant

3.6 Release of quercetin encapsulated by Ca-Ag/Ba-Ag/ Mg-Ag beads

The release of quercetin in the modified alginate matrix was analyzed using % release with two media,that is, pH 1.2 and pH 7.4, and then the variable effect of the type of crosslinking agent (Ca^{2+}, Ba^{2+} and Mg^{2+}) which is shown in Figure 8. The release of quercetin from the modified alginate matrix is influenced by the ability of the alginate to develop (swelling) (Sanchez-Ballester *et al*., 2019). Figure 7 shows that the % release of quercetin at pH 7.4 was higher than pH 1.2, so the swelling ratio of alginate was better than pH 1.2.

The pH conditions smaller than pKa, alginate, and alginic acid are strong because they form bonds that bind the encapsulated material and protect it from harsh environmental conditions (Goswami *et al*., 2014). It is known that gastric fluid media does not damage alginate nanoparticles because the pH conditions are below the pKa of alginate, thus providing stability to the nanoparticles and making them suitable carriers in oral formulations (Goswami *et al*., 2014).

A more targeted drug/active compound release occurs in the large intestine. Due to its therapeutic benefits, the large intestine has the primary function of absorbing water and salt from digested food and storing waste products until they are excreted. Another advantage is that the activity of proteolytic enzymes is relatively low compared to the small intestine (Prasanth *et al*., 2012). *Bacteroides*, *Bifidobacterium, Eubacterium*, *Peptococus, Lactobacillus*, and *Clostridium* produce various reductive and hydrolytic enzymes as secretions which are responsible for the degradation of di- and tri-polysaccharides through the breakdown of polymer strength. However, alginate is not degraded by human colonic bacteria (Agüero *et al.*, 2017).

3.7 Release kinetic of encapsulated quercetin in Ca-Ag/ Ba-Ag/Mg-Ag beads

The release of quercetin from alginate was influenced by pH conditions and the type of crosslinking agent. Therefore, further analysis related to the release of quercetin from alginate was carried out by calculating the value of the quercetin release kinetics constant on the variable pH and type of crosslinking agent. The kinetic models used in this study are the Zero order, Higuchi and Korsmeyer-Peppas models. The data of the kinetic model of the quercetin release in the modified alginate are presented in Table 1.

The results of the value of the relational constant (R^2) from Table 1 for the three kinetic models of Zero order, Higuchi and Korsmeyer-Peppas indicate that the R^2 value that is close to 1 is the Korsmeyer-Peppas model with the average R^2 value for all variables and the pH medium of quercetin release with matrix modified alginates (Ca-Ag, Ba-Ag, and Mg-Ag) 1.2 and 7.4 are 0.975. This results in the release of quercetin using a modified alginate matrix (Ca-Ag, Ba-Ag, and Mg-Ag) is more suitable and leads to the Korsmeyer-Peppas kinetic

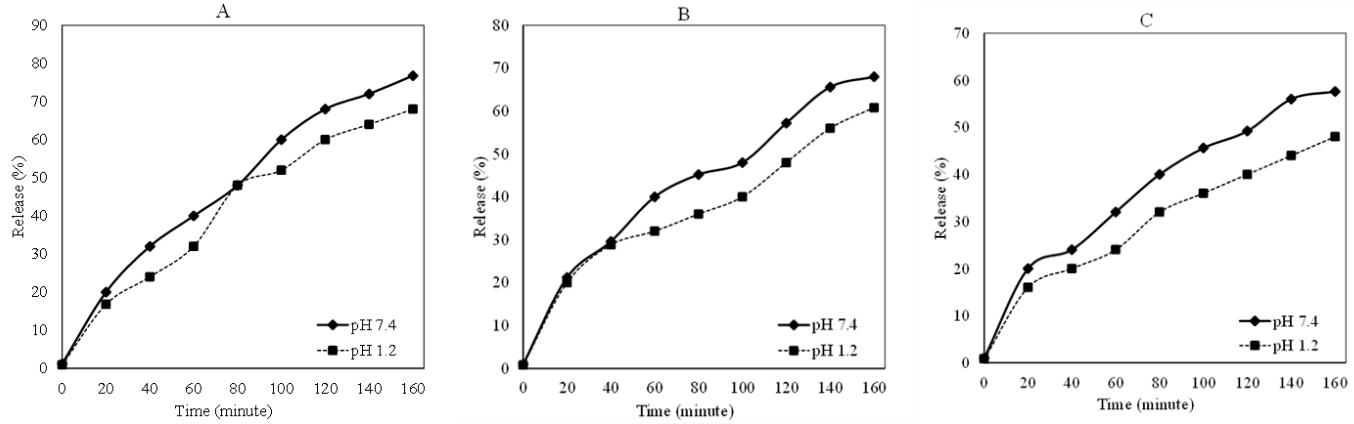

Figure 7. Effect of crosslinking agent and pH on the percentage of release of quercetin (A) Ca-Ag; (B) Ba-Ag; (C) Mg-Ag.

https://doi.org/10.26656/fr.2017.8(S1).10

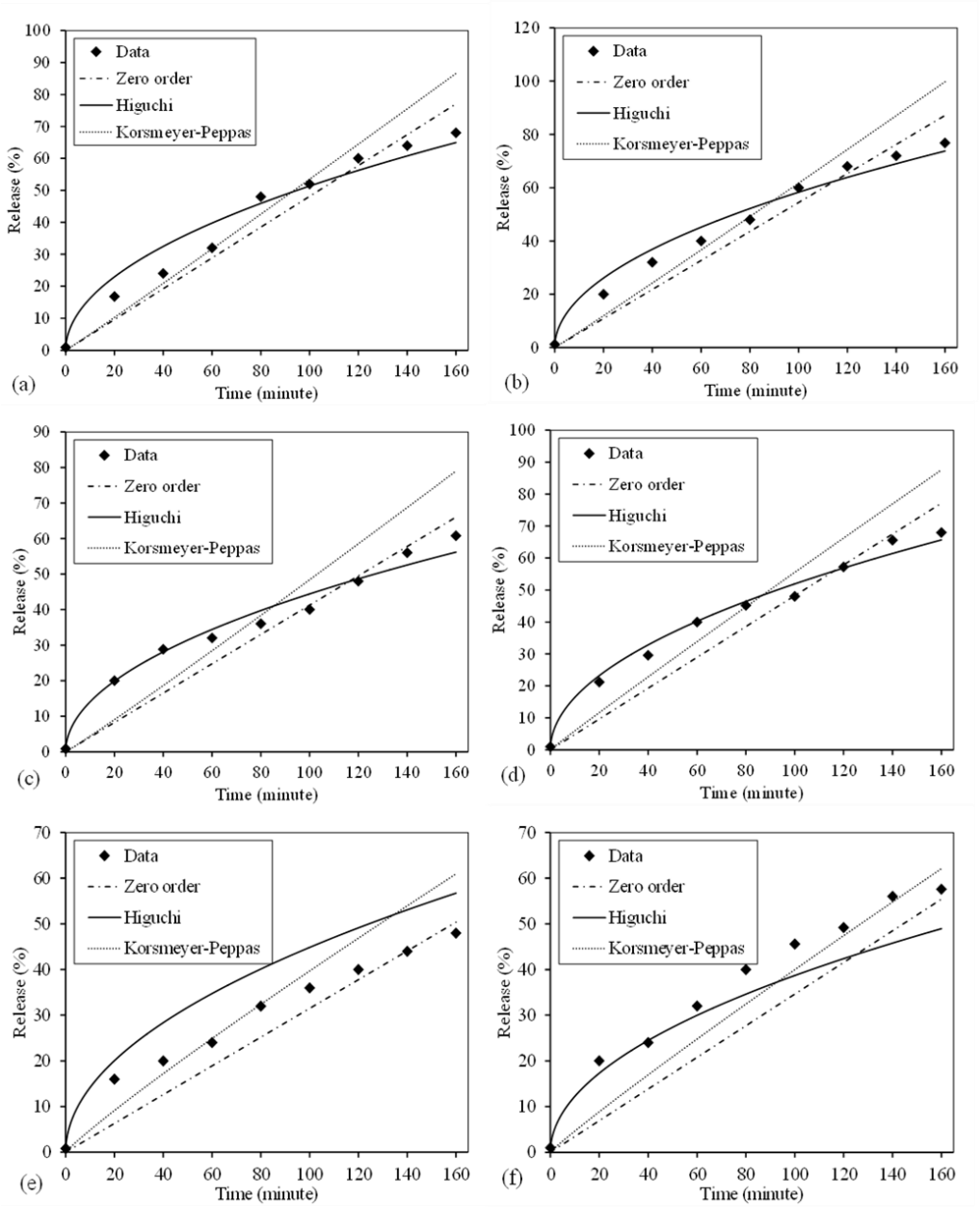

Figure 8. Fit of the experimental data of the percentage of release quercetin with kinetic model.

Table 1. Quercetin release kinetics models.

Crosslinking Agent	pH	Zero Order		Higuchi Model		Korsmeyer-Peppas Model		
		k_0	R^2	k_H	R^2	k_p	n	R^2
Ca/Ag	1.2	1.20×10^{-1}	0.934	1.2843	0.952	4.80×10^{-3}	1.024	0.992
	7.4	1.36×10^{-1}	0.919	1.4581	0.972	5.60×10^{-3}	1.021	0.990
Ba/Ag	1.2	1.03×10^{-1}	0.859	1.2983	0.973	4.00×10^{-3}	1.042	0.963
	7.4	1.20×10^{-1}	0.869	1.1107	0.985	6.40×10^{-3}	0.970	0.975
Mg/Ag	1.2	7.86×10^{-2}	0.881	1.1212	0.983	5.94×10^{-3}	0.912	0.976
	7.4	8.67×10^{-2}	0.861	0.9675	0.987	5.35×10^{-3}	0.937	0.975

model. The same results reported by several previous researchers, da Silva Fernandes *et al*. (2019) showed that the kinetic model of the crosslinking of Ca^{2+}, Zn^{2+} and Mn^{2+} alginates led to the Korsmeyer-Peppas kinetic model with an R^2 value of 0.984. Sariyer *et al.* (2020) have also carried out other research. In this study, alginate with a combination of carrageenan was used as a BSA protein encapsulation matrix by using the influence of the pH variable and the crosslinking agent. The types of ions used are Ca^{2+} and K^+. Therefore, a suitable kinetic model was applied to the release of BSA protein using a modified matrix of alginate and carrageenan with a crosslinked ion, namely Korsmeyer-Peppas, with a correlation coefficient value (R^2) of 0.995. Other researchers have also shown that using modified alginate with ionic crosslinking leads fits the Korsmeyer-Peppas kinetic model (Wardhani *et al*., 2021).

The Korsmeyer-Peppas kinetic model has a constant value of n, namely the diffusional exponent. According

https://doi.org/10.26656/fr.2017.8(S1).10

to Ritger and Peppas (1987) that the value of n resulting from the calculation of the Korsmeyer-Peppas kinetic model means the type of mechanism of diffusion of the encapsulated active ingredient. Beads with a spiral shape (round) can expand in media with a specific pH value. If the value of n is equal to or less than 0.43, it indicates Case I (Fickian diffusion); if n is equal to or greater than 0.85, the case II transport mechanism (polymer relaxation) predominates. In most cases, the released molecules with kinetic behavior depend on the diffusion and relaxation mechanisms' relative ratio, referred to as the transport anomaly, where the diffusion exponent, n, is between $0.43<n<0.85$. Table 1 shows the average n value is above 0.85. The same result was also conveyed by Sariyer *et al.* (2020).

4. Conclusion

Preparation of alginate modified by ionic crosslinking methods (Ca^{2+}, Ba^{2+} and Mg^{2+}) as a quercetin encapsulation matrix with the effect of crosslinking agent (Ca^{2+}, Ba^{2+} and Mg^{2+}) and pH. Swelling ratio and % solubility of the bead gel increased with time. The maximum swelling of 69 (g/g) and % solubility 36% at the Ca^{2+} as crosslinking agent. In this case, % EE of quercetin with Ca-Ag, Ba-Ag and Mg-Ag matrix reached 87%. The pH response of Ca-Ag, Ba-Ag, and Mg-Ag bead gel was investigated using pH 1.2 and 7.4. The concentrations of crosslinking agents $CaCl_2$, $BaCl_2$, $MgCl_2$ 0.4 M were selected to evaluate the study of quercetin release from the matrix. At pH 7.4, % release of quercetin gave the maximum yield of 78% with crosslinking agent $CaCl_2$, while at low pH (1.2), the release of quercetin did not occur sustainably. The suitable release kinetics study on quercetin was the Korsmeyer-Peppas model with a value of R^2 0.975 and a value of n 0.984 which indicates that the mechanism that occurs in quercetin release is a polymer relaxation transport mechanism. The success of methacrylate being covalently bound was proven by FTIR analysis, where a new peak appeared at 1271 cm^{-1}, it is the peak characteristic of the carbonyl group C-O-C group that is indicating quercetin loading in both Ca/Ag/Qe, Ba/Ag/Qe, and Mg/Ag/Qe encapsulated beads. Morphological analysis was carried out by SEM, Ca-Ag, Ba-Ag, Mg-Ag had a porous and rough surface. The results show that Ca -Ag, Ba-Ag, Mg-Ag beads can be successful as a drug delivery system for the control of quercetin release. This evidently established that the Ca-Ag, Ba-Ag, Mg-Ag bead gel can be exercised in medicine and applications related to pharmaceutics for further use in futures.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors would like to thanks the Diponegoro University for financial support of through RPIBT Grant Research Program Diponegoro University.

References

Agüero, L., Zaldivar-Silva, D., Peña, L. and Dias, M. (2017). Alginate microparticles as oral colon drug delivery device: A review. *Carbohydrate Polymers,* 168, 32–43. https://doi.org/10.1016/j.carbpol.2017.03.033

Ardiles, C.S. and Rodríguez, C.C. (2021). Theoretical study for determining the type of interactions between a GG block of an alginate chain with metals Cu^{2+}, Mn^{2+}, Ca^{2+} and Mg^{2+}. *Arabian Journal of Chemistry*, 14(10), 103325. https://doi.org/10.1016/j.arabjc.2021.103325

Barleany, D.R., Lestari, R.S.D., Yulvianti, M., Susanto, T.R. and Shalina, E. (2017). Acrylic acid neutralization for enhancing the production of grafted chitosan superabsorbent hydrogel. *International Journal on Advanced Science Engineering and Information Technology*, 7 (2), 702-708. https://doi.org/10.18517/ijaseit.7.2.2340

Bierhalz, A.C.K., da Silva, M.A., Braga, M.E.M., Sousa, H.J.C. and Kieckbusch, T.G. (2014). Effect of calcium and/or barium crosslinking on the physical and antimicrobial properties of natamycin-loaded alginate films. *Food Science and Technology,* 57(2), 494–501. https://doi.org/10.1016/j.lwt.2014.02.021

Cao, L., Lu, W., Mata, A., Nishinari, K. and Fang, Y. (2020). Egg-box model-based gelation of alginate and pectin: A review. *Carbohydrate Polymers*, 242, 116-389. https://doi.org/10.1016/j.carbpol.2020.116389

Chavoshpour-Natanzi, Z. and Sahihi, M. (2019). Encapsulation of quercetin-loaded β-lactoglobulin for drug delivery using modified anti-solvent method. *Food Hydrocolloids,* 96, 493–502. https://doi.org/10.1016/j.foodhyd.2019.05.051

Chuang, J.J., Huang, Y.Y., Lo, S.H., Hsu, T.F., Huang, W.Y., Huang, S.L. and Lin, Y.S. (2017). Effects of pH on the shape of alginate particles and its release behavior. *International Journal of Polymer Science*, 2017, 3902704. https://doi.org/10.1155/2017/3902704

da Silva Fernandes, R., Tanaka, F.N., de Moura, M.R. and Aouada, F.A. (2019). Development of alginate/starch-based hydrogels crosslinked with different ions: Hydrophilic, kinetic and spectroscopic properties. *Materials Today Communications*, 21,

100636. https://doi.org/10.1016/j.mtcomm.2019.100636

Dhanka, M., Shetty, C. and Srivastava, R. (2018). Methotrexate loaded gellan gum microparticles for drug delivery. *International Journal of Biological Macromolecules*, 110, 346–356. https://doi.org/10.1016/j.ijbiomac.2017.12.026

Draget, K.I., Skjåk-Bræk, G. and Stokke, B.T. (2006). Similarities and differences between alginic acid gels and ionically crosslinked alginate gels. *Food Hydrocolloids,* 20(2-3), 170–175. https://doi.org/10.1016/j.foodhyd.2004.03.009

Estevinho, B.N., Rocha, F., Santos, L. and Alves, A. (2013). Microencapsulation with chitosan by spray drying for industry applications – A review. *Trends in Food Science and Technology,* 31(2), 138–155. https://doi.org/10.1016/j.tifs.2013.04.001

Ezhilarasi, P.N., Indrani, D., Jena, B.S. and Anandharamakrishnan, C. (2014). Microencapsulation of Garcinia fruit extract by spray drying and its effect on bread quality. *Journal of Science Food Agriculture* 94, 1116–1123. https://doi.org/10.1002/jsfa.6378

Ferraz-Carvalho, R.S., Pereira, M.A. and Linhares, L.A. (2016). Effects of the encapsulation of usnic acid into liposomes and interactions with antituberculous agents against multidrug-resistant tuberculosis clinical isolates. *Jayasundera Journal of Microbiological Methods,*111, 330–334. https://doi.org/10.1590/0074-02760150454

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A. and Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Research International,* 40(9), 1107–1121. https://doi.org/10.1016/j.foodres.2007.07.004

Goswami, S., Bajpai, J. and Bajpai, A.K. (2014). Calcium alginate nanocarriers as possible vehicles for oral delivery of insulin. *Journal of Experimental Nanoscience*, 9(4), 337–356. https://doi.org/10.1080/17458080.2012.661472

Guarrotxena, N. and Quijada-Garrido, I. (2016). Optical and swelling stimuli-response of functional hybrid nanogels: feasible route to achieve tunable smart core shell plasmonic polymer nanomaterials. *Chemistry of Materials,* 28(5), 1402–1412. https://doi.org/10.1021/acs.chemmater.5b04517

Guo, J., Giusti, M.M. and Kaletunç, G. (2018). Encapsulation of purple corn and blueberry extracts in alginate-pectin hydrogel particles: impact of processing and storage parameters on encapsulation efficiency. *Food Research International*, 107, 414–422. https://doi.org/10.1016/j.foodres.2018.02.035

Hao, J., Guo, B., Yu, S., Zhang, W., Zhang, D., Wang, J. and Wang, Y. (2017). Encapsulation of the flavonoid quercetin with chitosan-coated nano-liposomes. *LWT -Food Science and Technology*, 85, 37–44. https://doi.org/10.1016/j.lwt.2017.06.048

Hasan, I., Shekhar, C., Alharbi, W., Khanjer, M.A., Khan, R.A. and Alsalme, A. (2020). A highly efficient ag nanoparticle-immobilized alginate-g-polyacrylonitrile hybrid photocatalyst for the degradation of nitrophenols. *Polymers*, 12(12), 3049. https://doi.org/10.3390/polym12123049

Heřmánková, E., Zatloukalová, M., Biler, M., Sokolová, R., Bancířová, M., Tzakos, A.G. and Vacek, J. (2019). Redox properties of individual quercetin moieties. *Free Radical Biology and Medicine*, 143, 240-251. https://doi.org/10.1016/j.freeradbiomed.2019.08.001

Haug, A. and Larsen, B. (1971). Biosynthesis of alginate. *Carbohydrate Research,* 17(2), 297–308. https://doi.org/10.1016/S0008-6215(00)82537-9

Hu, C., Lu, W., Mata, A., Nishinari, K. and Fang, Y. (2021). Ions-induced gelation of alginate: Mechanisms and applications. *International Journal of Biological Macromolecules,* 177, 578–588. https://doi.org/10.1016/j.ijbiomac.2021.02.086

Jana, L., Maity, P.P., Perveen, H., Dash, M., Jana, S., Dey, A. and Chattopadhyay, S. (2018). Attenuation of utero-toxicity, metabolic dysfunction and inflammation by soy protein concentrate in rats exposed to fluoridated water: consequence of *hyperlipidemia* in parallel with *hypohomocysteinemia. Environmental Science and Pollution Research*, 36, 36462-36473. https://doi.org/10.1007/s11356-018-3542-5

Jao, W.C., Chen, H.C., Lin, C.H. and Yang, M.C. (2009). The controlled release behavior and pH- and thermo-sensitivity of alginate/poly(vinyl alcohol) blended hydrogels. *Polymers for Advanced Technologies*, 20(8), 680–688. https://doi.org/10.1002/pat.1318

Karaman, K. (2019). Characterization of Saccharomyces cerevisiae based microcarriers for encapsulation of black cumin seed oil: stability of thymoquinone and antioxidant properties. *Food Chemistry,* 313, 126-129. https://doi.org/10.1016/j.foodchem.2019.126129

Lacerda, L., Parize, A.L., Fávere, V., Laranjeira, M.C.M. and Stulzer, H.K. (2014). Development and evaluation of pH-sensitive sodium alginate/chitosan microparticles containing the antituberculosis drug rifampicin. *Materials Science and Engineering C,* 39, 161–167. https://doi.org/10.1016/j.msec.2014.01.054

Lim, G.P. and Ahmad, M.S. (2017). Development of Ca-alginate-chitosan microcapsules for encapsulation and controlled release of imidacloprid to control dengue outbreaks. *Journal of Industrial and Engineering Chemistry*, 56, 382–393. https://doi.org/10.1016/j.jiec.2017.07.035

Mihaila, S.M., Gaharwar, A.K., Reis, R.L., Marques, A.P., Gomes, M.E. and Khademhosseini, A. (2012). Photo crosslink able kappa-carrageenan hydrogels for tissue engineering applications. *Advanced Healthcare Materials*, 2(6), 895–907. https://doi.org/10.1002/adhm.201200317

Nesterenko, A., Alric, I., Silvestre, F. and Durrieu, V. (2013). Vegetable proteins in microencapsulation: A review of recent interventions and their effectiveness. *Industrial Crops and Products,* 42, 469–479. https://doi.org/10.1016/j.indcrop.2012.06.035

Petrova, V.A., Elokhovskiy, V.Y., Raik, S.V., Poshina, D.N., Romanov, D.P. and Skorik, Y.A. (2019). Alginate gel reinforcement with chitin nanowhiskers modulates rheological properties and drug release profile. *Biomolecules,* 9(7), 291. https://doi.org/10.3390/biom9070291

Pool, H., Mendoza, S., Xiao, H. and Mc Clements, D.J. (2013). Encapsulation and release of hydrophobic bioactive components in nanoemulsion-based delivery systems: impact of physical form on quercetin bioaccessibility. *Food Function*, 4(1), 162–174. https://doi.org/10.1039/C2FO30042G

Prasanth, R., Aravindan, V. and Srinivasan, M. (2012). Novel polymer electrolyte based on cob-web electrospun multi component polymer blend of polyacrylonitrile/poly(methyl methacrylate)/ polystyrene for lithium ion batteries—Preparation and electrochemical characterization. *Journal of Power Sources,* 202, 299–307. https://doi.org/10.1016/j.jpowsour.2011.11.057

Raja, A., Waldecker, L. and Zipfel, J. (2019). Dielectric disorder in two-dimensional materials. *Nat. Nanotechnology,* 14, 832–837. https://doi.org/10.1038/s41565-019-0520-0

Ramdhan, T., Ching, S.H., Prakash, S. and Bhandari, B. (2019). Time dependent gelling properties of cuboid alginate gels made by external gelation method: Effects of alginate-$CaCl_2$ solution ratios and pH. *Food Hydrocolloids*, 90, 232–240. https://doi.org/10.1016/j.foodhyd.2018.12.022

Ritger, P.L. and Peppas, N.A. (1987). A simple equation for description of solute release ii. Fickian and anomalous release from swellable devices. *Journal of Controlled Release,* 5(1), 23-36. https://doi.org/10.1016/0168-3659(87)90034-4

Sanchez-Ballester, N.M., Soulairol, I., Bataille, B. and Sharkawi, T. (2019). Flexible heteroionic calcium-magnesium alginate beads for controlled drug release. *Carbohydrate Polymers*, 207, 224–229. https://doi.org/10.1016/j.carbpol.2018.11.096

Sarıyer, S., Duranoğlu, D., Doğan, Ö. and Küçük, İ. (2020). pH-responsive double network alginate/ kappa-carrageenan hydrogel beads for controlled protein release: Effect of pH and crosslinking agent. *Journal of Drug Delivery Science and Technology*, 56, 101551. https://doi.org/10.1016/j.jddst.2020.101551

Shang, Y., Ding, F., Liu, J., Xiao, L., Deng, H., Du, Y. and Shi, X. (2017). Dual-drug release from chitin-based core-shell microspheres fabricated by coaxial electrospray. *Advances in Polymer Technology,* 37 *(*5), 1366-1373. https://doi.org/10.1002/adv.21795

Sriamorn-sak, P., Thirawong, N. and Korkerd, K. (2007). Swelling, erosion and release behavior of alginate-based matrix tablets. *European Journal of Pharmaceutics and Biopharmaceutics*, 66(3), 435–450. https://doi.org/10.1016/j.ejpb.2006.12.003

Tátraaljai, D., Major, L., Földes, E. and Pukánszky, B. (2014). Study of the effect of natural antioxidants in polyethylene: Performance of β-carotene. *Polymer Degradation and Stability,* 102, 33–40. https://doi.org/10.1016/j.polymdegradstab.2014.02.012

Topuz, F., Henke, A., Richtering, W. and Groll, J. (2012). Magnesium ions and alginate do form hydrogels: A rheological study. *Soft Matter*, 8(18), 4877–4881. https://doi.org/10.1039/c2sm07465f

Wardhani, D.H., Aryanti, N., Aziz, A., Firdhaus, R.A. and Ulya, H.N. (2021). Ultrasonic degradation of alginate: A matrix for iron encapsulation using gelation. *Food Bioscience*, 41, 100803. https://doi.org/10.1016/j.fbio.2020.100803

Zeng, F., Li, T., Gao, Q., Liu, B. and Yu, S. (2018). Physicochemical properties and in vitro digestibility of high hydrostatic pressure treated waxy rice starch. *International Journal of Biological Macromolecules,*120, 1030-1038. https://doi.org/10.1016/j.ijbiomac.2018.08.121

