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TRAPPING OF BETANIN IN ALGINATE MICROCAPSULES: STABILITY STUDIES UNDER ACCELERATED CONDITIONS

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Abstract

Microcapsules including red beet extract rich in betanin were produced by ionic gelation. Sodium alginate was used as wall material. Impacts of coating amount and active material were assessed with encapsulation efficiency (EE) of the capsules in terms of total phenolic content (TPC). Encapsulation of the red beet extract in alginate microbeads was satisfying with >80% under the best conditions of ionic gelation (15% calcium chloride concentration and 2% sodium alginate for 15 min of hardening time). The interaction between the active material and the alginate beads was also investigated by diffuse reflectance fourier transform (DRIFT) spectroscopy. Scanning electron microscopy (SEM) was used to comprehend the morphology. Shape properties were determined with sphericity factor (SF) and the roundness (Rn) by stereo-microscope. Accelerated oxidation test was employed to measure the stability of the microcapsules by Rancimat method. Stability of the microbeads were evaluated in terms of kinetic and thermodynamic analyzes.

Key words: biopolymers, encapsulation, particle morphology, phenolic compounds

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1. Introduction

Oxidation is one of the major reasons for food deterioration, which reduces the shelf-life of the foodstuff together with many damages (Sanches-Silva et al., 2014) (Fig. 1). On the other hand, the food's role has been directed towards preventing foodborne diseases, and improving the physical and mental prosperities of consumers currently. Attention in biologically active materials has been continuing to be strengthened by the development of advanced technologies as well as the research efforts driven by public interest and consumer demands to find out their activities and applications (Đorđević et al., 2014). Biologically active compounds exist in millions of plant, animal, marine organism and microorganism species, which can be obtained by a variety of methods (extraction and biotechnological). These high added-value ingredients can be used to prepare smart foods by increasing the shelf-life, nutritional value and consumer approval. Studies associate these valuable substances with numerous benefits on human health (Kim et al., 2016). Inclusion of bioactive compounds into food products provides many advantages in food protection, and subscribes to functional food improvement (Gülmez and Şahin, 2019; Kurtulbaş et al., 2018; Şahin et al., 2020). However, it is important to remember that direct application of these natural products to the products can lead to deterioration of the taste of the product. Additionally, direct application can be also resulted in loss of the function of the bioactive component (Silva-Weiss et al., 2013). It might also cause a decrease in the quality of functional food products by interacting with other

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ingredients in the food matrix. In order to overcome those drawbacks of addition of antioxidants directly into the food systems, microencapsulation has been a promising preservation method (Riseh et al., 2022).

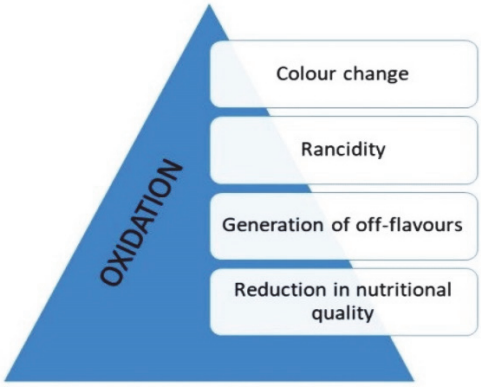


Fig. 1. Oxidation problems in food products

Betanin is an alternative natural additive in food, cosmetic and pharmaceutical products. It has a code (EEC No. E 162) given by European Union (Da Silva et al., 2019). It also has exceptionally strong scavenging activity against reactive oxygen species (Esatbeyoglu et al., 2015). Previously, after betanin was extracted from red beet, freeze drying and spray drying processes were applied to make microcapsules coated by maltodextrin and xanthan gum (Antigo et al., 2018). The same capsules were also used in yogurt product after produced by freeze drying and spray drying methods (Antigo et al., 2020). Recently, spray drying method was employed to prepare microcapsules coated with maltodextrin and sweet potato starch (Esquivel-González et al., 2022). Fathordoobady et al. (2021) reported that ionic gelation with alginate was a better way to encapsulate the betacyanin-type compounds such as betanin due to their sensitivity towards heat.

In this study, commercial red beet extract containing betanin was supplied as active substance to be trapped by microbeads. Biopolymers have been recommended as wall materials for encapsulation processes as wall material to create microbeads (Moradi-Pour et al., 2021; Riseh et al., 2021a). Alginate, which has been known as valuable for producing biodegradable beads was used as coating material in the current study (Riseh et al., 2021b). It is one of the most consumed polymeric matrices in order to trap the active material from releasing (Fathi et al., 2021a; Fathi et al., 2021b; Moradi Pour et al., 2022; Pour et al., 2019; Pour et al., 2022; Riseh et al., 2022). Hence, several concentrations of alginate solutions (1%, 1.5 %, and 2%, w/v) were used as wall material.

Active materials of red beet extract were trapped into alginate microbeads by ionic gelation encapsulation method. The stability of the microcapsules against oxidation was measured by means of accelerated oxidation test (Rancimat method). The Rancimat findings also have provided the calculation of the kinetics and thermodynamic parameters of the investigated product (De Sousa et al., 2019; Gülmez and Şahin, 2019).

Furthermore, DRIFT (diffuse reflectance Fourier transform) spectroscopy was used to investigate the interaction between the active material and the alginate beads. The morphology of the beads was also analyzed by scanning electron microscopy (SEM) technology. Additionally, shape properties of capsules were also defined with sphericity factor and the roundness.

2. Materials and methods

Fig. 2 summarizes the experimental work comprehensively.

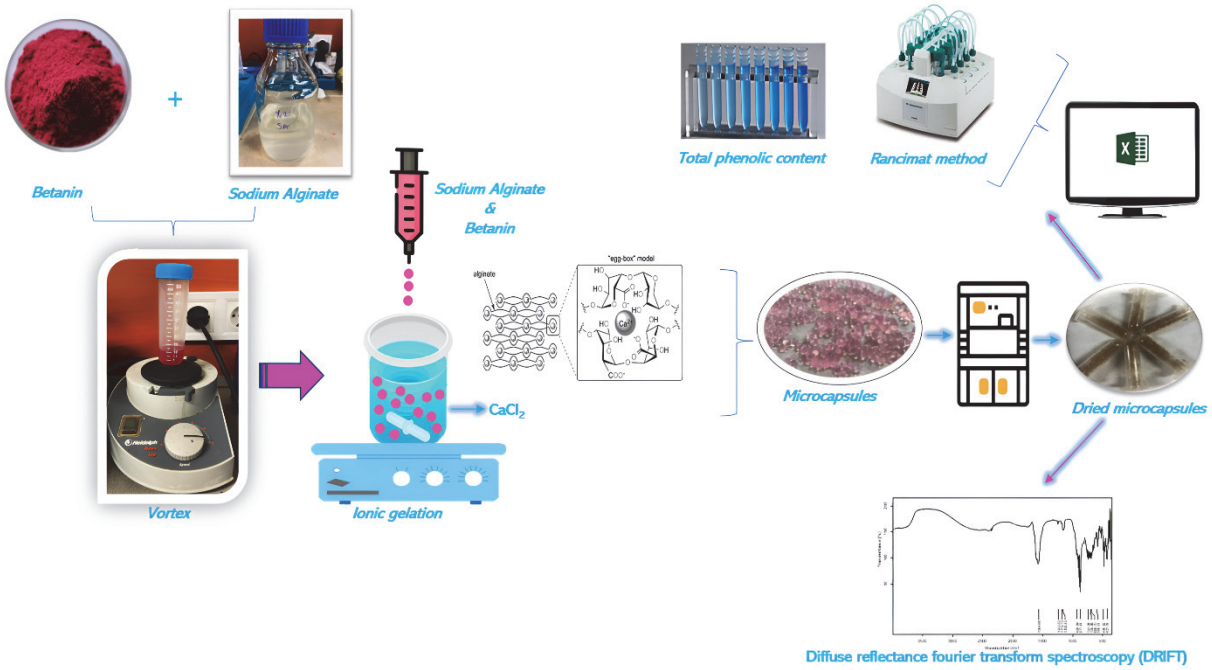


Fig. 2. Summary of the experimental method

2.1. Materials

Betanin (red beet extract diluted with dextrin), Folin-Ciocalteu reagent, sodium alginate, sodium carbonate, gallic acid monohydrate and calcium chloride dihydrate ≥ 97 (ISOLAB) were provided from Sigma Aldrich (St. Louis, MO, USA). Ethanol, methanol and polyethylene glycol were supplied from Merck (Darmstadt, Germany).

2.2. Ionic gelation

Ionic gelation method was employed for the encapsulation of betanin in alginate beads. Briefly, red beet extract rich in betanin was active core material. Calcium chloride was used as gelling medium, while sodium alginate was used as wall material. Three different ratios of sodium alginate (1% w/v, 1.5% w/v and 2% w/v) solutions were prepared. Alginate solutions were prepared by dissolving the required amount of powdered alginate in distilled water, with the alginate ratio in the final solution being 10 g/L, 15 g/L and 20 g/L, respectively. The prepared solutions were kept at +4°C for 24 hours before usage in order to remove air bubbles formed during mixing.

Calcium chloride solutions used in the gelling medium were prepared at a certain concentration (15%, w/v). As a result of dissolving a certain amount of calcium chloride 2-hydrate ($CaCl_2.2H_2O$) in distilled water, the $CaCl_2$ concentration was prepared to be 150 g/L. After betanin (0.05 g and 0.10 g) was added to sodium alginate solutions (1% w/v, 1.5% w/v, 2% w/v), the resulting mixtures were mixed by vortex for 3 minutes. Several ratios of betanin to sodium alginate solution was performed. The mixture was dropped by a 5 mL syringe into $CaCl_2$ solution (15% w/v) on a magnetic stirrer (270 rpm) (Model MSH-20D, DAIHAN Scientific Co. Ltd., Korea) under ambient conditions. After the capsules were hardened in the gelling medium for a certain period of time (15 min) at ambient temperature, the beads were filtered. Microbeads were washed 3 times with distilled water before drying. The microbeads were dried at ambient temperature under the fume hood for 24 h.

2.3. Bioactivity measurements

To determine the total phenolic content (TPC) of the capsules, approximately 0.1 g of microcapsules were weighed, and dissolved in 3 mL of ethanol:acetic acid:water (50:8:42 v/v/v) (Tolun et al., 2016). This mixture was fractionated by a homogenizer (IKAT25, ULTRA-TURRAX) at 7000 rpm for 1 min, and filtered using 0.45 µm syringe filters. The Folin-Ciocalteau method was used to determine the total phenolic compounds. In this method, the Folin-Ciocalteau reagent is reduced with sodium carbonate in the presence of phenolic substances. Folin-Ciocalteu reagent, which is yellow in color, turns blue as a result of oxidation of phenolic compounds in basic media (Toprakçı et al., 2022). 100 µL of the microcapsule mixture was taken into 2000 µL of Folin-Ciocalteau reagent (10%, v/v). After this mixture was kept in the dark for 5 min, 1800 µL of sodium carbonate solution (7.5%, w/v) was added into the mixture, and mixed. After the resulting mixture was kept in the dark for 1 hour, absorbance values were recorded at 765 nm via a UV spectrophotometer (PG Instruments, T60 / Leicestershire, England). Results were given in mg gallic acid equivalent (mg-GAE) per liter (ppm).

2.4. Surface phenolic content of capsule

To determine the surface phenolic content (SPC) of the capsules, approximately 0.1 g of microcapsules were weighed, and dissolved in 3 mL of ethanol and methanol mixture (1:1, v/v). This mixture was fractionated by a homogenizer at 7000 rpm for 1 min, and filtered using 0.45 µm syringe filters. The amounts of surface phenolic compounds of the capsules were measured and quantified by the same method described as above. Similarly, SPC findings were given in ppm unit.

2.5. Encapsulation efficiency

Microencapsulation efficiency was calculated by using the surface and total phenolic compounds in the microcapsules. Encapsulation efficiency (EE) is the ratio of the encapsulated phenolic content to the total phenolic content. The encapsulated phenolic content was determined by taking the difference between the total phenolic content (TPC) and the surface phenolic content (SPC). The encapsulation efficiency of the microcapsules was calculated according to (Eq. 1):

$$EE(\%) = \frac{TPC - SPC}{TPC} \cdot 100 \quad (1)$$

2.6. Oxidation stability of the microcapsules

Accelerated oxidation test was employed to measure the stability of the microcapsules against oxidation. Rancimat method was applied by means of Rancimat 892 device (Metrohm, 892, Herisau, Switzerland). Approximately 100 mg of capsule samples were placed in the tubes of the device. The air flow rate was set as 20 L per hour. The device was operated under 4 different temperature conditions (110, 120, 130 and 140 °C), respectively. Time of resistance against oxidation in the samples was given as the induction time (IT).

2.7. Kinetic and thermodynamic approaches

Arrhenius equation (Eq. 2) was applied due to the dependence of the physicochemical reactions on the temperature (Jafari et al., 2017).

$$Ink = InA - \frac{Ea}{R} \times \frac{1}{T} \quad (2)$$

k = reaction rate constant (h^{-1})
A = frequency factor
E_a = activation energy (kJ mol^{-1})
R = universal gas constant (8.314 J mol^{-1} K^{-1})
T = temperature (K)

Thermodynamics parameters such as enthalpies (ΔH^o) and entropies (ΔS^o) of the oxidation reaction were computed by using (Eq. 3) derived from the transition state theory (Eyring equation):

$$In\frac{k}{T} = \left[\left(\frac{k_B}{h}\right) + \left(\frac{\Delta S^\circ}{R}\right)\right] - \left(\frac{\Delta H^\circ}{R}\right) \cdot \left(\frac{1}{T}\right) \quad] \quad (3)$$

K_B = Boltzman constant ($1.38x10^{-23}$ J K^{-1})
H = Planck constant ($6.63x10^{-34}$ J s)

2.8. Diffuse reflectance Fourier transform spectroscopy (DRIFT)

Bruker Alpha-T DRIFT spectrometer with a 528/D model through OPUS 6.5 software (Bruker Optics Inc.) was used for the characterizations of the samples. Potassium bromide was added to the powder samples before they were pressed into pellets. The wavelength range was between 400 and 4000 cm^{-1}.

2.9. *Particle morphology and size of capsules*

Capsules morphology obtained at optimum process conditions was measured by stereo-microscope (Stemi 2000-C, Zeiss, Germany) and AxioCamERc5 camera (Stemi 2000-C, Zeiss, Göttingen, Germany). The measurements of the capsules were carried out using the Zeiss (Carl Zeiss Microscopy GmbH, Germany) program. Determination of shape properties of capsules are defined as how close to sphericity with sphericity factor (SF) and the roundness (Rn). Briefly, capsules diameter obtained at optimum process conditions was measured, and calculated by following equations (Eqs. 4-5) (Lee et al., 2013):

$$Spherical\ factor\ (SF) = \frac{d_{\max} - d_{\min}}{d_{\max} + d_{\min}} \quad (4)$$

$$Roundness\ (Rn) = \frac{p^2}{4\pi A} \quad (5)$$

d_{max}=*Maximum diameter*
d_{min}=*Minimum diameter*
P=*Perimeter*
A= *protection area*

2.10. Scanning electron microscopy

SEM analysis was carried out to determine the morphological properties of capsules. The samples were visualized on the Zeiss LEO 1430 model SEM (Germany) device after being coated with a 10 nm gold and palladium layer.

3. Results and discussions

3.1. Microencapsulation of betanin in alginate hydrogels

Stability of microencapsulated active substance has been also investigated by means of accelerated oxidation test (Rancimat method) (Navarro-Flores et al., 2020). The stability of the capsules against oxidation was given as induction time. On the other hand, induction time was selected as dependent variable in order to identify the amount of active material and wall material for the preparation of microcapsules (Table 1). The stability of the capsules increased in terms of induction time as the alginate ratio increased. Similar observations were also reported in the encapsulation of lemon balm extract with ionic gelation (Najafi-Soulari et al., 2016). Increasing the sodium alginate concentration enhanced the efficiency in terms of polyphenol yield in the alginate hydrogels containing lemon balm extract. Rise in the alginate ratio increases the thickness of the membrane of the capsule, which gives rise to prevent the escape of the active material into the solution (Blandino et al., 1999). Wissam and Samer (2019) also reported similar alginate effect on the efficiency of hydrogels containing flaxseed oil extract (Wissam and Samer, 2019). They explained this outcome with the fact that sodium alginate amount favoured active Ca binding locations in the polymer chain owing to the higher degree of crosslinking. Similarly, Zam et al. (2014) also observed the same effect on the yield of alginate beads containing pomegranate peel antioxidants. Encapsulation efficiencies with respect to TPC and proanthocyanidins were enhanced by increasing the alginate amount from 1% to 3% (w/v). Efficiency of the polyphenols and proanthocyanidins increased from 3% to 33 and 29 %, respectively. Hence, the more the polysaccharide wall material, the greater the protection of the active material in the bead structure. Xu et al. (2022) observed that the stability of betanin (encapsulated in mesoporous silica nanoparticles) was significantly improved by coating the structure with chitosan-sodium tripolyphosphate layer. On the other hand, Serris and Biliaderis (2001) monitored the stability by periodic absorption measurements with spectrophotometric methods. They also reported the significance of some factors (pH, temperature, light, air and presence of pro-oxidant metal ions) on the stability of the microcapsules. Additionally, Vergara et al. (2014) explained the degradation of the betanin microparticles produced by spray drying depending on the microparticles hygroscopicity.

When the amount of active material is considered, increasing the amount of the extract enhanced the resistance to oxidation (Table 1). This is expectable, since there is a correlation between the

antioxidative phenolic components and the oxidative stability. Actually, this positive relationship occurs due to the fact that phenolic materials deliver their hydrogen atoms to stop the oxidation reaction.

Encapsulation efficiency was 80.373% under the best conditions of ionic gelation (15% calcium chloride concentration and 2% sodium alginate for 15 min of hardening time). Tumbas Šaponjac et al. (2016) reported a close EE to ours, where they used freeze drying method with soy protein as coating. Amjadi et al. (2018) reported almost the same EE (80.35%) for the betanin loaded in liposomal nanocarriers. Shafqat et al. (2023) also achieved 87.5% encapsulation efficiency for the same active substance loaded in chitosan nanoparticles. Poornima and Sinthiya (2017) used spray drying for the encapsulation of the betanin rich extract with more than 96% of EE.

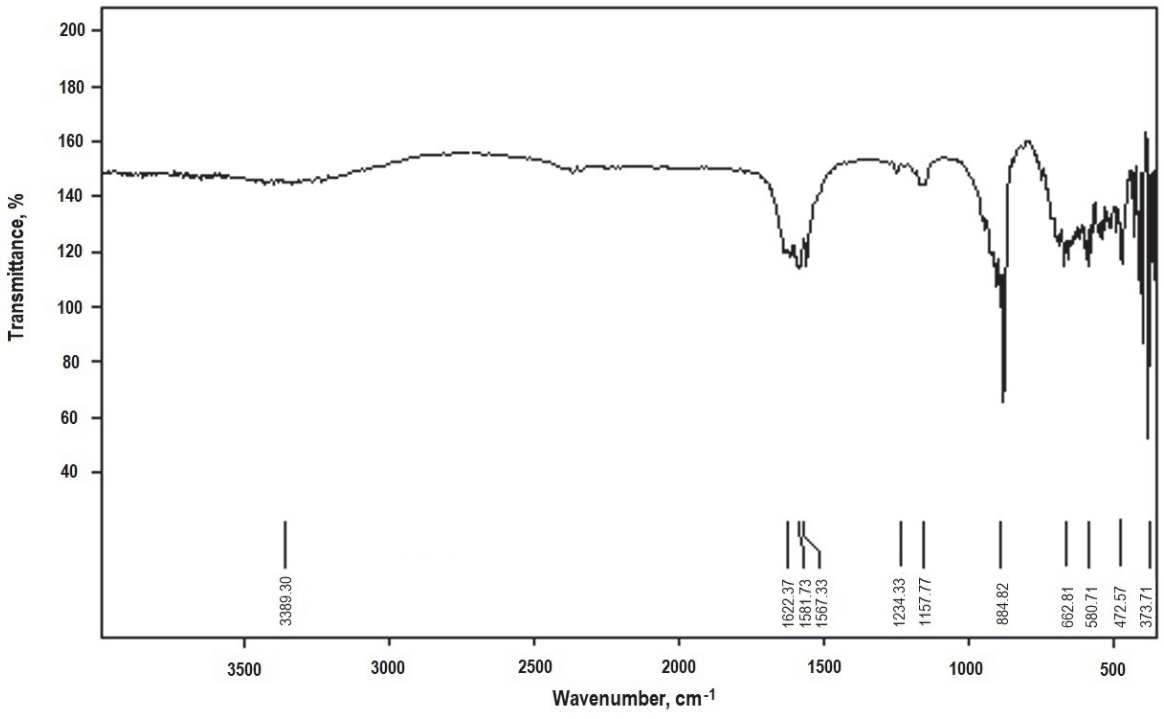
Similarly, increasing the concentration of the wall material enhanced the EE. Čakarević et al. (2020) applied two different methods (freeze drying and spray drying) to produce capsules. EE of the spray drying was less than the current study (75%), whereas 92% of EE was achieved by freeze drying method. This finding shows that the yield of the encapsulation process is affected by the procedure significantly. Additionally, spray drying requires elevated temperature, which results in the degradation problems in heat sensitive components such as betacyanin-type compounds.

3.2. DRIFT findings

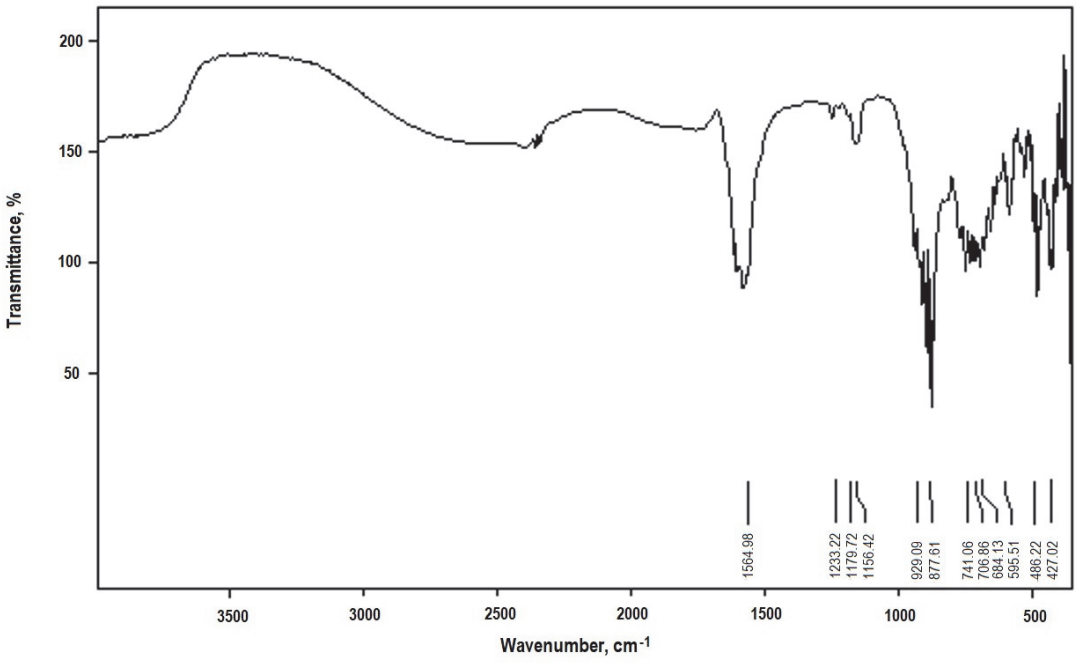
Fig. 3 is the DRIFT spectrums of the red beet extract and the alginate capsules containing the extract. Fig. 3.a shows the spectrum of betanin rich extract, where there is absorption band at 1622 cm^{-1} refers to C=N stretching. Additionally, the peaks around 1234 cm^{-1} are attributed to C-O of the carboxylic acid vibrations, while C-COOH bond stretching are seen at the absorption band around 884 cm^{-1} (Devadiga and Ahipa, 2020). Considering the capsule (Fig. 3b), there are asymmetric and symmetrical stresses due to -COO- (carboxylic group) in the peaks of the capsule at 1564 and 1417 cm^{-1}. The peaks at 900-1200 cm^{-1} show -O-C-O- stretches of ether groups and -C-O- stretches of alcohol groups (Okur et al., 2018). Saccharide ring of the capsule appears on the peak around 1150 cm^{-1} (Najafi-Soulari et al., 2016). The characteristic peaks of the active material still exist in the alginate beads, which verifies the non-interaction between the active material and the coating material. This finding indicates the success of the selected encapsulation process (López Córdoba et al., 2013).

Table 1. Comparative results of sodium alginate and active material amount in terms of induction time

Sample	*Red been extract (g)*	*Sodium alginate (%, w/v)*	*Induction time (h)*
1	0.10	1.0	0.63±0.02
2	0.10	1.5	0.72±0.03
3	0.10	2.0	1.19±0.04
4	0.05	1.0	0.59±0.01
5	0.05	1.5	0.68±0.01
6	0.05	2.0	0.74±0.02



(a)



(b)

Fig. 3. IR spectrum of (a) red beet extract and (b) alginate bead containing red beet extract

3.3. Particle size and shape

Fig. 4 presented that the image of betanin alginate beads measured with the Zeiss program. The size of the alginate beads ranged from ~0.19 mm to ~0.29 mm (mean diameter), while the SF and Rn values were calculated as 0.06±0.02 and 1.12±0.01, respectively. Alginate-based hydrogel beads capsulated tea polyphenols diameter were found in range between 2.12-2.66 mm (Li et al., 2021). In a study the thyme aqueous extract was encapsulated with alginate bead methods, the average diameter of freeze-dried capsules was 0.44 mm, while the diameter of the oven-dried samples was 0.63 mm (Stojanovic et al., 2012). *Rn* values indicate a particle for a perfect sphere to approaching infinity and the more perfectly spherical the capsules are closer to 1. On the other hand, *SF* was indicating the deformation factor and values were close to 0 for a perfect sphere (Lee et al., 2013; Lević et al., 2015). According to Chan et al. (2009), the SF value defines the difference in the shapes of the capsules into four basic types: egg-shaped, pear-shaped, tear-shaped and spherical. The shape of the beads is highly dependent on factors such as alginate concentration, dripping height and speed (Chan et al., 2009).

The SEM results show that alginate beads containing betanin had spherical image with a relatively smooth surface, while the shrinkage structure was formed due to drying after encapsulation process (Fig. 5). In a study, thyme extract was encapsulated with alginate beads method, it was stated that different drying methods were quite effective on the morphology of alginate beads, and more smooth capsules were obtained by drying in the oven rather than freeze drying (Stojanovic et al., 2012).

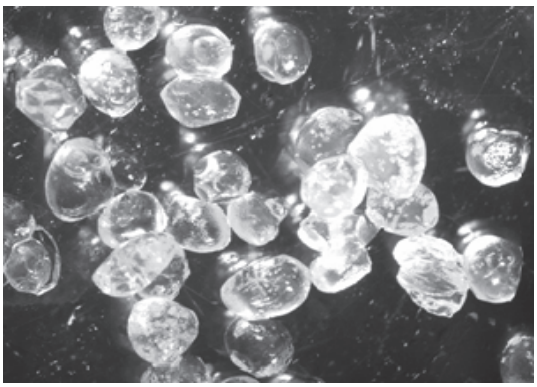


Fig. 4. Microphotograph of the alginate bead containing red beet extract

3.4. Kinetic and thermodynamic findings

Table 2 gives the kinetic parameters for the oxidation process of the microbeads. Expectedly, temperature enhanced the oxidation reaction rate constant. k is assumed as the induction time's reverse due to the first order reaction. When the activation energy is considered, our E_a amount is relatively smaller than those of the literature (Jafari et al., 2017; Pérez-Alonso et al., 2003). Low activation energy values (< 40 kJ mol^{-1}) denote to the oxidation processes which are not heat sensitive. This means that the concerned oxidation reaction is not affected by only temperature (Galvan et al., 2013).

Table 3 shows the ΔH^{++}, ΔS^{++} and ΔG^{++} values. ΔH^{++} is known as the energy, which is exhausted while the oxidation reactions are occurred (Spacino et al., 2015). To be more clear, the enthalpy is a measure of the energy barrier that must be overcome (Kragović et

al., 2019). Positive enthalpy change (6.751 kJ mol^{-1}) signs to an endothermic system. On the other hand, formation of irregularity is represented by ΔG^{++}. In this study, relatively low entropy value is attributed to more stable product with less energy (<40 kJ mol^{-1}) (Garvín et al., 2017).

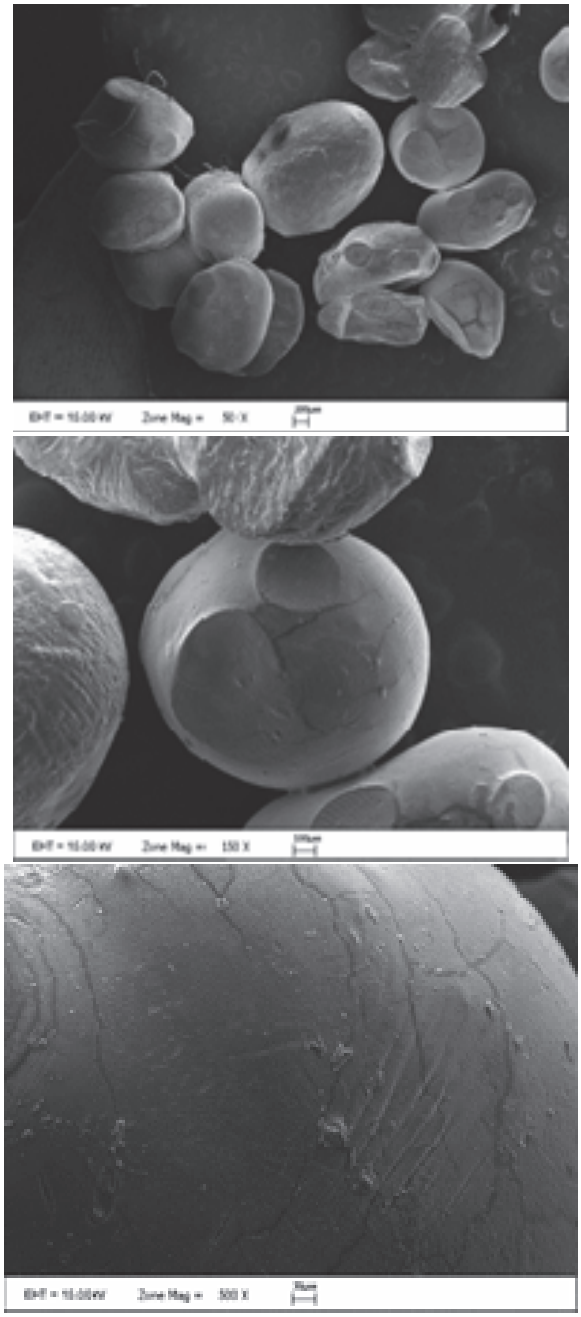


Fig. 5. Scanning electron microscopy images of the alginate bead containing red beet extract

A positive change of Gibbs free energy (100.012, 101.586, 104.061 and 106.536 kJ mol^{-1}) is ascribed to an endergonic and non-spontaneous system, which is not approving indeed (Spacino et al., 2015). This outcome is also supported by negative entropy change together with positive enthalpy change. Kuhn et al. (2022) also reported the disorder change of the molecules in the system as negative during the degradation of *Bougainvillea glabra* bracts extract capsules, while the entahlpy change was positive. To conclude, our microparticles have demonstrated high stability at high temperatures depending on the kinetic and thermodynamic findings.

Table 2. Kinetic parameters for the stability of the alginate beads including red beet extract

Parameter	***Microcapsules***
k (h^{-1}) at 110 ºC	0.7605
k (h^{-1}) at 120 ºC	0.7905
k (h^{-1}) at 130 ºC	0.8065
k (h^{-1}) at 140 ºC	0.8333
E_a (kJ mol^{-1})	3.87
R^2	0.9882

Table 3. Thermodynamic parameters for the stability of the alginate beads including red beet extract

Parameter	***Microcapsules***
ΔG^{++}(kJ mol^{-1}) at 383.15 K	101.586
ΔG^{++} (kJ mol^{-1}) at 393.15 K	104.061
ΔG^{++} (kJ mol^{-1}) at 403.15 K	106.536
ΔG^{++} (kJ mol^{-1}) at 413.15 K	100.012
ΔH^{++} (kJ mol^{-1})	6.751
ΔS^{++} (J K^{-1} mol^{-1})	-0.248
R^2	0.9505

4. Conclusions

In the present study, ionic gelation method has been used for the encapsulation of red beet extract in alginate beads. The stability of the capsules increased in terms of induction time as the alginate ratio and the active material increased. The highest encapsulation efficiency in terms of total phenolic substance (80.373%) was achieved by 15% calcium chloride concentration and 2% sodium alginate for 15 min of hardening time.

Based on the DRIFT findings, there was no interaction between the active material and the coating material, verifying the feasibility of the proposed encapsulation formulation. The SEM images showed that the alginate bead capsules obtained at optimum conditions were spherical and smooth. In addition, the SF and Rn values confirm that the obtained alginate bead capsule is very close to sphericity.

Low activation value of the Arrhenius model shows a chemically unstable process. ΔH^{++}, ΔS^{++} and ΔG^{++} values denote to a less favorable system, which is endothermic, endergonic and non-spontaneous.

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