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Research Article

Hydrolyzed Karaya Gum: Gelatin Complex Coacervates for Microencapsulation of Soybean Oil and Curcumin

My Lan Nguyen Le, Hang Nga Le Thi, and Vinh Tien Nguyen

Faculty of Chemical and Food Technology, Ho Chi Minh City University of Technology and Education, 01 Vo Van Ngan, Thu Duc District, Ho Chi Minh City, Vietnam

Correspondence should be addressed to Vinh Tien Nguyen; tiennv@hcmute.edu.vn

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This is the first report on utilizing hydrolyzed karaya gum (HKG) as a novel polyanion material for complex coacervation with gelatin A. With negative zeta potentials at pH > 2.5, HKG formed the complex coacervate with a maximum yield at pH 3.75 and 1 : 1 HKG:gelatin ratio. The optimal complex coacervates were used to encapsulate soybean oil containing curcumin using different shell:core ratios, homogenization speeds, concentrations of emulsifier, and drying techniques. Optical microscopy showed that increasing homogenization speed and Tween 80 concentration produced smaller and more uniform coacervate particles. Increasing the shell:core mass ratio from 1 to 4 resulted in a linear increase in encapsulation efficiencies for both soybean oil and curcumin. Accelerated peroxidation tests on the microcapsules showed enhanced protective effects against oil peroxidation when increasing shell:core ratios and using freeze-drying instead of oven-drying at 50 °C. *In vitro* release of curcumin in simulated gastric and intestinal fluids was faster when using freeze-drying and decreasing shell:core ratio. This study shows perspective novel applications of HKG in microencapsulating active ingredients for food and pharmaceutical industries.

1. Introduction

Karaya gum (KG) is the dried exudate from *Sterculia urens* of the family Sterculiaceae. It is a branched, acidic, and partially acetylated polysaccharide with high molecular mass (9–16 MDa) composed of neutral monosaccharides (13–26% galactose and 15–30% rhamnose) and sugar acids (30–43% galacturonic with less than 6% glucuronic). The main chain is composed of 0–4 units of α-D-galacturonic acid and 0–2 units of L-rhamnose. The side chains are linked to the main chain by (1⟶2)-β-D-galactose and a small content of (1⟶3)-β-D-glucuronic acid bounded to galacturonic acid units [1]. KG occurs naturally as a salt of Ca^{2+} and Mg^{2+} [2].

Due to the water/moisture absorbing, gel- and film-forming, and adhesive properties, KG is used in hair-dressing lotions and finger wave lotions in cosmetics, as an emulsifier, stabilizer, or thickener in the food industry, as a bulk laxative and dental adhesive in medicine [3]. Native KG can be used as a polymer substrate in packaging films containing antibacterial components to improve food shelf-life [4–6]. Drugs can be loaded on KG-based blends [1, 7–10], or KG-based hydrogels grafted with other polymers [11–13]. Responsive hydrogels based on KG grafted with organic and inorganic polymers are widely studied for dye adsorption applications [14–19].

The main barrier of KG to wider applications in the food industry is its low solubility caused by the presence of acetyl groups (≈8%) and divalent cations as crosslinkers (Ca^{2+}, Mg^{2+}) [20]. Therefore, deacetylation by alkaline hydrolysis is a simple chemical modification of KG to increase its solubility and widen its application range. To the best of our knowledge, although having many useful properties including high viscosity, high molecular mass, and emulsifying and stabilizing ability, hydrolyzed karaya gum (HKG) is not widely studied and utilized. We found only reports about further esterification of HKG with dodecenyl succinic or maleic anhydride to produce antibacterial modified KG [21, 22].

Containing galacturonic acid residues, HKG can exist as a polyanion and interact with other polycations to form polyelectrolyte complexes called complex coacervates. The formation of these coacervates, known as complex coacervation, is usually used to encapsulate, protect, and controllably release unstable and active ingredients in foods and drugs [23, 24]. Gelatin is one of the most popular polycations studied for complex coacervation due to its non-toxicity, high emulsifying and stabilizing capacities, high crosslinking ability through amino groups, abundance, and low price [25]. Gelatin is manufactured by hydrolysis of collagen under acidic (gelatin A) or basic (gelatin B) conditions. Gelatin A has an isoelectric point (pI) of nearly 9.2, while pI of gelatin B is approximately 5. At pH < pI, gelatin has net positive charges and can interact and form complex coacervates with polyanions, such as gum arabic [26, 27], pectin [28], sodium alginate [29], agar [30], sodium carboxymethyl cellulose [31], chia mucilage [32], and seed gums [33]. Edible oils are a common object for microencapsulation in the food industry because they are sensitive to oxidation due to a high content of C=C bonds in their molecules [34]. Curcumin is a bioactive ingredient isolated from turmeric powder. Despite its wide range of biological activity, including antioxidant, antimicrobial, antiviral, anti-inflammatory, antidiabetic, and anticancer properties, curcumin suffers from low solubility in water, low bioavailability, and poor pharmacokinetic profiles [35]. To overcome these disadvantages, curcumin was microencapsulated using spray-drying [36], freeze-drying [37], isoelectric precipitation [38], or complex coacervation [39, 40].

In this study, we investigated the complex coacervation between HKG and gelatin A and then used the complex coacervate to encapsulate soybean oil and curcumin. We studied the influences of pH and biopolymer ratios on the formation of the complex coacervate to find optimal conditions. We then further studied the influences of homogenization speed, emulsifier concentration, shell:core ratios, and drying techniques on properties of microcapsules, including size and morphology, encapsulation efficiency, oxidative stability of soybean oil, and release of curcumin in simulated gastric and intestinal fluids.

2. Materials and Methods

2.1. Chemicals. KG (Xuan Hong brand) and soybean oil were purchased in a local supermarket in Ho Chi Minh city. Absolute ethanol, HCl (35–38%), NaOH, CH_3COONa, KH_2PO_4, KSCN, Mohr salt, NaCl, Tween 80, ethyl acetate, petroleum ether, and gelatin A (bloom 150) were purchased with analytical grades from Xilong Scientific (China), pepsin 1 : 3000 from Titan Biotech (India), and pancreatin from Now (USA). Curcumin was synthesized according to a patent and purified by thrice recrystallization in ethanol [41].

2.2. Methods

2.2.1. Alkaline Hydrolysis of KG. KG was hydrolyzed according to a published method [42]. KG (2 g) was ground to powder, added to 100 mL of water, and left for hydration at room temperature under stirring (200 rpm) in 24 h. A solution of 1 M NaOH (33 mL) was added to the KG suspension. The mixture was stirred for 30 min at 200 rpm, then neutralized with 1 M HCl, and left under stirring for 30 min at room temperature. Absolute ethanol (90 mL) was added to precipitate the hydrolyzed gum. The precipitated HKG was collected and washed twice with 75% v/v ethanol, cut into small pieces, dried at 50°C for 24 h, ground into powder, and stored in a polyethylene bag in a refrigerator at 4°C for further uses.

2.2.2. Zeta Potential of HKG and Gelatin. Zeta potentials of 0.1% w/v HKG and gelatin solutions were measured at 25°C with a Zetasizer Nano ZS90 at various pH adjusted by using 0.1 M HCl or 0.1 M NaOH.

2.2.3. Influence of pH and HKG:Gelatin Ratio on Complex Coacervation. To study the influences of pH and HKG:gelatin mass ratio on the complex coacervation, we adapted the experimental setups from another paper [43]. Each biopolymer (0.5 g) was dissolved in 100 mL of distilled water to obtain a stock 0.5% w/v solution. These solutions were mixed with volume ratios of 4 : 1, 2 : 1, 1 : 1, 1 : 2, and 1 : 4 (HKG:gelatin) and stirred for 5 min. Because the initial w/v concentrations of both biopolymers are equal, the volume ratio of their solutions is also the mass ratio of polymers. The pH of each mixture was adjusted under stirring with 0.1 M HCl or 0.1 M NaOH. The absorbance at 600 nm of each mixture at each pH was measured using a spectrophotometer (UH5300, Hitachi, Japan). The pH with maximum absorbance for each HKG : gelatin ratio was considered optimal for the complex coacervation.

Because a high absorbance does not necessarily associate with ease in solid recovery by centrifugation, we further studied the influence of HKG:gelatin ratios on the recovery percentages of the complex coacervates. The mixtures above with different HKG:gelatin ratios were adjusted to their optimal pH, then left for 24 h for complete coacervation, and centrifuged at 4500 rpm for 10 min to recover the solid coacervates. The solids were dried at 105°C until unchanged mass. The recovery of HKG-gelatin complex coacervates was calculated using

$$\text{Recovery}\,(\%) = \frac{m_c}{m_g + m_{\text{HKG}}} \cdot 100, \tag{1}$$

where m_c is the mass of the dried HKG-gelatin coacervate and m_g and m_{HKG} are the initial masses of gelatin and HKG used in each stock solution.

2.2.4. Encapsulation of Soybean Oil Containing Curcumin Using HKG-Gelatin Complex Coacervates. Microcapsules with shell:core ratios of 1 : 1, 2 : 1, and 4 : 1 were prepared based on another method with slight modifications [44]. The complex coacervate shell was composed of HKG and gelatin with a fixed ratio of 1 : 1. HKG and gelatin were dissolved separately to obtain 0.5% w/v solutions. Due to the basic nature of HKG solution, its pH was pre-adjusted to 7 to

prevent the degradation of curcumin in basic media. Ethyl acetate (1 mL) was used to dissolve 25 mg of curcumin and then mixed with 25 mL of soybean oil. After vortex, the mixture was added to the gelatin solution. Tween 80 was then added with different mass ratios compared to the oil core (0%, 0.5%, or 1.0%). The mixture was homogenized (Ultra-Turrax 25, IKA, Germany) for 5 min at different speeds (3000, 6000, or 9000 rpm). The neutralized HKG solution was added to the mixture under homogenization with the same speed used above. After homogenization, the pH was adjusted to 3.75 using 1 M HCl. The mixture was then stirred for 3 h at 10 ± 1°C for the formation of complex coacervates and then centrifuged at 4500 rpm for 10 min to obtain the solid microcapsules.

The wet microcapsules were then dried using two methods: (i) prefreezing at −30°C for 8h and then freeze-drying (FD) under vacuum (0.05 mbar) at −5°C for 24 h and then at 25°C for 24 h using a Stellar lyophilizer (Millrock Technology, USA); (ii) forced-draft oven-drying (OD) at 50°C for 24 h using a Memmert SF55 oven (Germany).

The composition of 1 L of final mixture for each shell: core ratio is given in Table 1.

2.2.5. Optical Microscopy. Before drying, the dispersions of soybean oil-curcumin encapsulated in HKG-gelatin coacervate were diluted 10-fold and observed at 1000X magnification using an optical microscope (CX33, Olympus) connected with a computer.

2.2.6. Determination of Encapsulation Efficiencies (EE) of Soybean Oil and Curcumin. The encapsulation yields of soybean oil and curcumin were determined by measuring the amount of oil and curcumin on the surface and inside the dried microcapsules [45].

To quantify the oil and curcumin on the surface of microcapsules, 0.5 g of microcapsules was dispersed in 20 mL of petroleum ether and stirred for 10 min. The solid was filtered on a filter paper and washed with 3 × 10 mL of petroleum ether. The filtrate and washing ether were combined and evaporated at room temperature, and then at 105°C for 1 h, and weighed.

The remaining solid was left in the air to remove the petroleum ether residue, then mixed, and shaken with 5 mL of absolute ethanol for exactly 3 min to dissolve curcumin on the surface of microcapsules. The mixture was then filtered, and the solid was washed with 3 × 2 mL of ethanol. The filtrate and washing ethanol portions were combined and made up to 25 mL with ethanol in a volumetric flask. The absorbance at 424 nm of the solution was measured and used to calculate the amount of the surface curcumin using a curcumin standard curve in ethanol (equation 2):

$$\text{Absorbance} = 0.1514\,\text{C}\ (\mu\text{g/mL}) + 0.0639\ \left(\text{with } R^2 = 0.9989\right). \tag{2}$$

The solid remaining after surface curcumin determination was left in the air for drying for 1 h. Then, 10 mL of 4 M HCl was added and the mixture was stirred for 2 h to break the shell of microcapsules and release the oil and curcumin. After that, 10 mL of petroleum ether was added and the mixture was shaken vigorously for 10 min to extract the released oil. The mixture was then centrifuged at 2500 rpm for 30 min. The upper ether layer was taken out and dried to determine the amount of released oil. The extraction with petroleum ether was repeated one more time to ensure complete oil extraction.

Table 1: Amounts of components used in 1 L of final solution for microencapsulation of soybean oil and curcumin.

Shell:core ratio	HKG (g)	Gelatin (g)	Curcumin (mg)	Soybean oil (g)
1 : 1	2.50	2.50	5.0	5.00
2 : 1	2.50	2.50	2.5	2.5
4 : 1	2.50	2.50	1.25	1.25

The remaining solid and liquid layer after centrifugation was added with 10 mL of ethyl acetate and shaken for 10 min to extract curcumin. After centrifugation and taking out the upper layer containing curcumin, the process of curcumin extraction was repeated two more times to ensure complete curcumin extraction. The extracted ethyl acetate portions were combined and made up to 50 mL with ethyl acetate. The absorbance at 424 nm of the solution was measured and used to calculate the amount of encapsulated curcumin using a curcumin standard curved in ethyl acetate (equation 3):

$$\text{Absorbance} = 0.0964C\,(\mu\text{g/mL}) - 0.018\ \left(\text{with } R^2 = 0.9991\right). \tag{3}$$

Encapsulation efficiencies (EE) for oil and curcumin were calculated using equations (4) and (5):

$$\text{EE}_{\text{oil}}\,(\%) = \frac{m_{\text{so}}}{m_{\text{eo}} + m_{\text{so}}} \times 100, \tag{4}$$

$$\text{EE}_{\text{cur}}\,(\%) = \frac{m_{\text{sc}}}{m_{\text{ec}} + m_{\text{sc}}} \times 100, \tag{5}$$

where m_{so} and m_{eo} are the weights of surface and encapsulated fractions of oil, respectively, and m_{sc} and m_{ec} are the weights of surface and encapsulated fractions of curcumin, respectively.

2.2.7. Oxidative Stability of Encapsulated Soybean Oil. Ferric thiocyanate method was used to evaluate the accelerated oxidation of the encapsulated and the control soybean oil [46]. The microcapsules and the control oil were placed in Petri dishes in an oven at 105 C. After every 3 h, 0.1 mL of the control oil was vigorously mixed with 0.1 mL of Tween 20, 9.7 mL of distilled water. After that, 20 μL of 30% KSCN and 20 μL of 20 mM Mohr salt $(NH_4)_2Fe(SO_4)_2$ in HCl 3.5% were added and shaken for 5 min. The absorbance of the mixture at 500 nm was measured as an indication of the amount of peroxide formed. Before measuring the oxidation extent of encapsulated oil, the microcapsules were stirred with 5 mL of 4 M HCl for 2 h to break the shell and release the encapsulated oil. Petroleum ether was added to extract the oil and then evaporated. The extent of

peroxidation of the oil was tested by the procedure used for the control. The measurements were conducted until the absorbance of the control reached a maximum.

The percentage of inhibition of peroxidation for encapsulated oil was calculated using

$$\text{peroxidation inhibition (\%)} = \frac{A_c - A_e}{A_c} \times 100, \quad (6)$$

where A_c is the maximum absorbance from the control and A_e is the absorbance from the encapsulated oil when the control reached maximum absorbance.

2.2.8. In Vitro Release of Curcumin. The release of curcumin from microcapsules was studied *in vitro* using simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) [47].

The total amount of curcumin on the surface and inside 1.0 g of microcapsules was determined by sequentially digesting the microcapsules with 20 mL of 4 M HCl for 2 h under stirring, extracting curcumin with ethyl acetate for three times (3 × 5 mL), combining the ethyl acetate layers, making up to 20 mL, and measuring the absorbance (A_o) at 424 nm.

SGF was prepared by dissolving 0.2 g of NaCl, 0.32 g of pepsin, 0.7 mL of 35–38% HCl in water, then making up 100 mL and adjusting to pH 2.0. The microcapsules (1.0 g) were stirred in the SGF for 2 h at 37°C. Ethyl acetate (15 mL) was then added and the mixture was vigorously shaken for 5 min to extract curcumin and then centrifuged at 3000 rpm for 5 min. The upper layer was taken out, made up to 20 mL with ethyl acetate, and the absorbance at 424 nm was measured (A_{SGF}). The percentage of curcumin released in SGF was calculated as $(A_{SGF}/A_o) \cdot 100\%$.

SIF was prepared by dissolving 0.68 g of KH_2PO_4, 0.62 g NaOH, and 1.0 g of pancreatin in distilled water, then making up to 100 mL and adjusting to pH 7.0. The microcapsules (1.0 g) were stirred in 50 mL of SGF for 2 h, and then 50 mL of SIF was added. The mixture was stirred again for 4 h. The released curcumin was quantified by extracting with ethyl acetate (3 × 5 mL), making up to 20 mL, and measuring the absorbance at 424 nm (A_{SIF}), as described above. The percentage of curcumin released in SIF was calculated as $(A_{SIF}/A_o) \cdot 100\%$.

To evaluate the effect of pepsin, the same procedures were carried out without adding pepsin in the SGF.

2.2.9. Statistical Analyses. All experiments were randomized and triplicated. The results are presented as mean ± standard deviation. Statistical significance of data was analyzed using Duncan's multiple range tests with the common criteria $p < 0.05$.

3. Results and Discussion

3.1. Zeta Potentials of HKG and Gelatin. To determine the optimal pH for the complex coacervation between HKG and gelatin, their zeta potentials were measured in solutions with different pH from 2.5 to 8.5 (Figure 1).

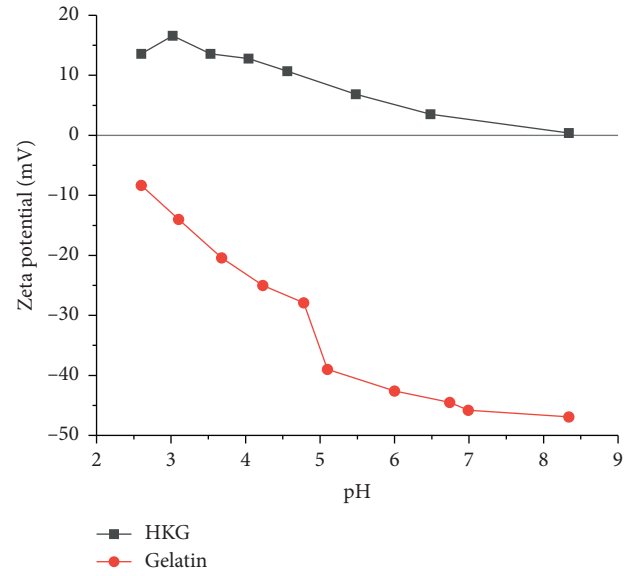

Figure 1: Zeta potentials of 0.1% gelatin and 0.1% HKG aqueous solutions at different pH.

Zeta potential is the measure of electrical potential and charges near the hydrodynamic slip plane of a microparticle. HKG had negative charges in the studied pH range because 37–40% of HKG are galacturonic and glucuronic acids [1]. Even in acidic solutions, the carboxyl groups in these acid units partially dissociate to carboxylate ions ($-COOH \longrightarrow -COO^- + H^+$), hence giving HKG the negative charges [48]. When the pH increases, these carboxyl groups of HKG are deprotonated to a larger extent and hence shift the zeta potentials to the negative side.

On the other hand, gelatin A has an isoelectric point of 9.2, making its molecules positively charged in the studied pH range lower than 9.2 [49]. Gelatin A is manufactured by partial acidic hydrolysis of collagen, so its molecule contains more free amino groups than free carboxyl groups, thus making it positively charged in acidic solutions.

The opposite charges of HKG and gelatin ensure the formation of polyelectrolyte complex between them in a wide pH range from acidic to weakly basic. When pH > 9.2, both polymers obtain negative charges and cannot form the complex. We expected the complex coacervation to be most effective at pH = 3–4, when the zeta potentials of HKG and gelatin are approximately equal, leading to a complex with almost no residue charge. The zero total charges would eliminate the electrostatic repulsions between the complex coacervate particles, hence promoting their agglomeration and recovery.

3.2. Influence of pH on HKG-Gelatin Complex Coacervation. The extent of HKG-gelatin complex coacervation was evaluated by the absorbance of the dispersion at 600 nm (turbidity) measured at different pH and HKG:gelatin ratios (Figure 2).

When pH of the dispersion decreased from 4.5 to 3.8, the turbidity of all HKG-gelatin dispersions increased. The reason

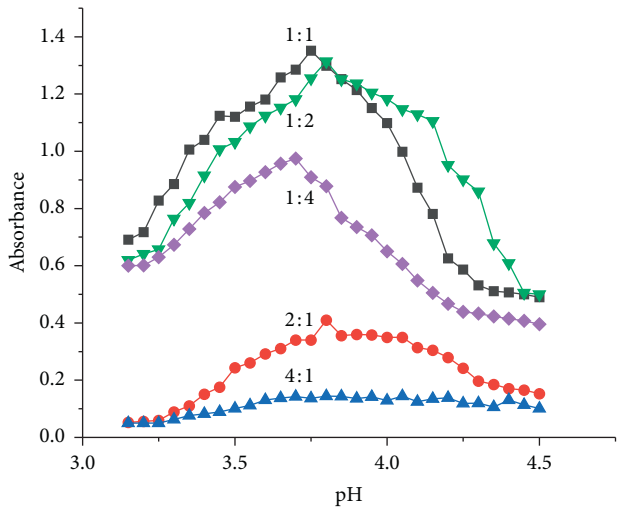

Figure 2: Turbidity of the complex coacervate dispersions at different pH and HKG:gelatin ratios.

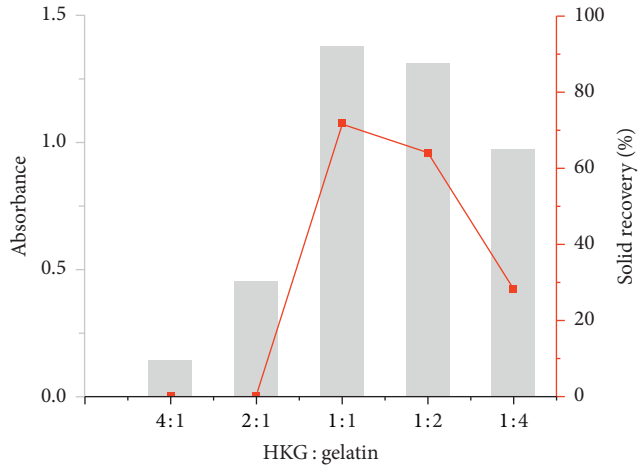

Figure 3: Maximum absorbance and solid recovery from the dispersions with different HKG:gelatin ratios.

for this trend is that when the dispersion became more acidic, the zeta potential of gelatin became more positive, while the zeta potential of HKG also increased but still in the negative region. The result was the total charge of the complex coacervates became less negative and thus reduced electrostatic repulsions between particles and the stability of HKG-gelatin dispersions. Zero-charged complex particles were formed at pH 3.7–3.8 and easily precipitated out of the solution, resulting in the maximum turbidities. This was the optimum pH region for the formation of HKG-gelatin complex coacervates, which is very close to the optimum pH for complex coacervation between gelatin and other polyanion biopolymers at 1 : 1 ratio, including acacia gum (3.75) [50], pectin (3.8) [51], sodium alginate (3.8–4.0) [29, 52], and cashew gum (4.1) [53].

However, when pH was lower than 3.7, the turbidity decreased (Figure 2), indicating the dissolution of the formed HKG-gelatin complex. When the medium is highly acidic, the HKG-gelatin complex becomes positively charged and this charge triggers the electrostatic repulsions between the complex particles, making them colloidally stable.

Figure 3 shows that the maximum turbidity and the amount of solid complex coacervates obtained after centrifugation were highest at the HKG:gelatin ratio of 1 : 1.

When gelatin was used at higher ratios (HKG:gelatin ratios of 1 : 2 and 1 : 4), the turbidity and complex recovery slightly decreased because some of the excessive gelatin would absorb onto the surface of HKG-gelatin complex and impart the complex a positive charge. Complex particles with positive charges repulse each other, keeping their sizes small and stable against centrifugation. Notably, when HKG was used at higher ratios (HKG:gelatin ratios of 2 : 1 and 4 : 1), the turbidity significantly decreased and even no solid was obtained after centrifugation. We found that the dispersions with high HKG contents are highly viscous, which might be the additional reason preventing HKG-gelatin complex particles to agglomerate and settle under centrifugation. In summary, we chose pH 3.75 and HKG:gelatin mass ratio 1 : 1 as the optimal conditions for the maximum formation and recovery of HKG-gelatin complex.

3.3. Microscopy Analysis. Technologically, it is desired that the HKG-gelatin complex particles are small and homogeneous. Therefore, homogenization and emulsifier Tween 80 were used to reduce the particle size and stabilize the formed particles, respectively. The dispersions of HKG-gelatin microcapsules containing soybean oil and curcumin were 10-fold diluted and observed at 1000x magnification. The inset in Figure 4 shows that the HKG-gelatin complex coacervates were liquid-like spherical because HKG is a weak polyanion [54]. Although the droplets were in contact with each other, they did not unite into larger units, indicating a good encapsulating and protecting effect of the HKG-gelatin coacervate shell.

Increasing the homogenization speed from 3000 to 6000 rpm resulted in decreases in the mean and standard deviation of particle sizes ($n = 500$), indicating that the microcapsules were torn into smaller and more monodispersed particles. However, when the homogenization speed increased from 6000 to 9000 rpm, the mean of particle sizes decreased not significantly, and their standard deviation was almost unchanged. Therefore, 6000 rpm is an appropriate homogenization speed for the microencapsulation.

The concentration of emulsifier is another important factor regulating the particle sizes. When no emulsifier was used, the microcapsules were large with approximately 85% of their sizes ranging from 25 to 45 μm (Figure 5).

Although homogenization effectively reduced the sizes of oil droplets and the complex coacervates, the absence of an emulsifier resulted in their quick aggregation and larger particle sizes. The presence of Tween 80 decreased the surface tension between the particles and the aqueous medium, thus facilitating the breakup of the particles and preventing their coalescence [55, 56]. Increasing the concentration of Tween 80 also decreased the standard deviation

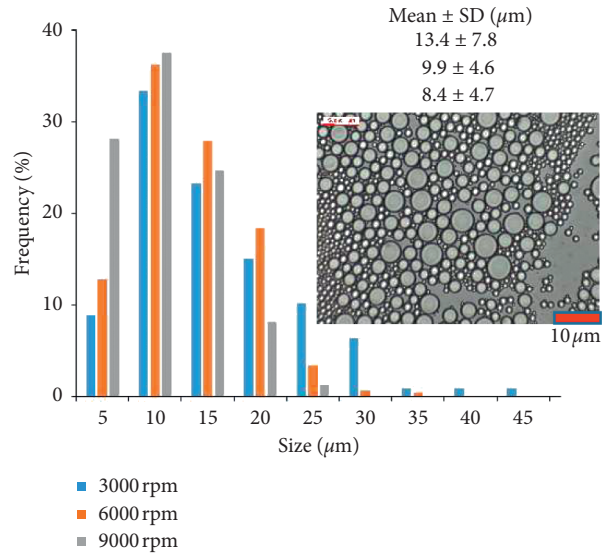

Figure 4: Influence of homogenization speed on the size distribution of HKG-gelatin coacervates. Inset: an example of optical microscopic images used for size analyses.

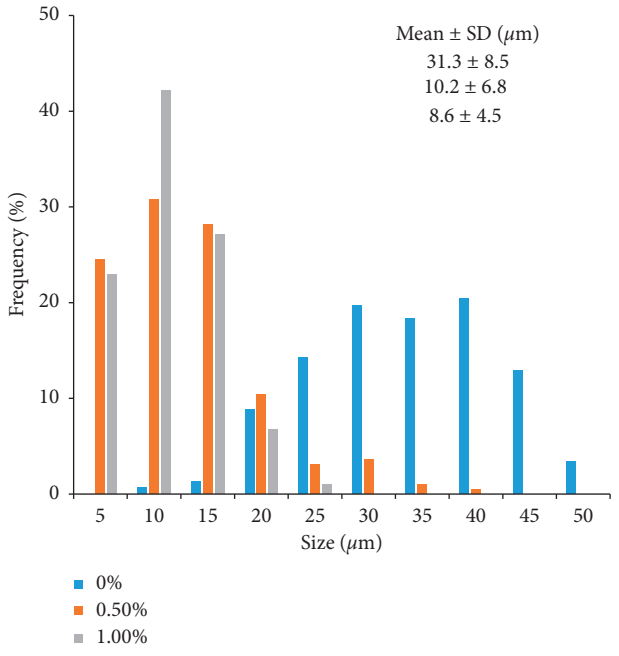

Figure 5: Influence of Tween 80 concentration on the size distribution of HKG-gelatin complex particles.

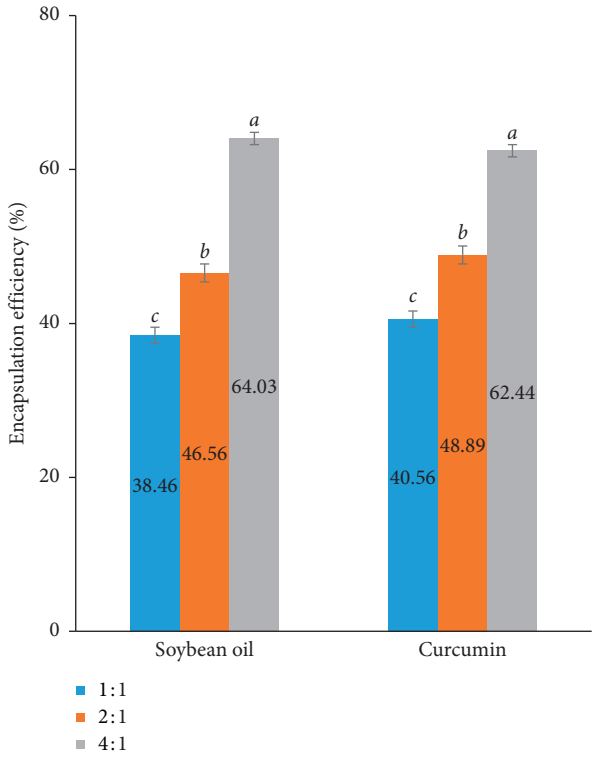

Figure 6: Encapsulation efficiencies of soybean oil and curcumin in microcapsules with different shell:core ratios.

(SD) of the coacervate sizes, indicating a more narrow size distribution.

3.4. Encapsulation Efficiencies for Soybean Oil and Curcumin. Figure 6 shows no significant differences ($p > 0.05$) between EE of soybean and curcumin at the same shell:core ratio. This indicates that curcumin was well contained in soybean oil and did not leak out to the aqueous medium due to the extremely low solubility of curcumin in water.

Increasing the shell:core ratio resulted in an increase in EE, which was in accordance with other studies on several complex coacervate shell:core systems, such as gelatin-acacia: vitamin A palmitate [26], gelatin-alginate:black pepper essential oil [29], and gelatin-chia mucilage:oregano essential oil [32]. Detailed calculations show that increasing one part of the shell (from 1 : 1 to 2 : 1) improved EE by approximately 8%

while increasing two parts of the shell (from 2 : 1 to 4 : 1) improved EE by 20%. This means that, in the studied shell: core range, every part of shell encapsulated approximately 8–10% of soybean oil and curcumin, indicating the oil was still in excess, the shell encapsulated at its maximum capacity, and there was still room for significantly higher EE by further increasing the shell:core ratio [57].

When increasing shell:core ratio from 1 : 1 to 2 : 1 and 4 : 1, the recovery of initial materials (gelatin, HKG, and oil) significantly ($p < 0.05$) increased from 48.5% to 55.6% and 68.1%, respectively. This increase was also linear with the increase in the shell amount in the encapsulating system, indicating that further increasing shell:core ratio may enhance solid recovery, beside EE. However, too high shell:core ratio would result in a very low release of curcumin in the intestinal fluid, as would be shown below in Section 3.6.

3.5. Oxidative Stability of Encapsulated Soybean Oil. One of the most important properties of edible oils is their oxidation to a series of products their causing off-flavours and rancidity. Peroxides are the products of the first steps in oil rancidity. We studied the protective effect of the HKG-gelatin shell against soybean oil peroxidation in an accelerated oxidative test using ferric thiocyanate method to evaluate the extent of oil peroxidation. The original soybean oil was used as the control. Figure 7 shows that, in the beginning, the OD microcapsules had higher degrees of oxidation than FD microcapsules. This is because, during OD at high temperature (50°C), the oil on the surface of microcapsules was oxidized at a higher extent than during FD under low temperature and in vacuum.

After an induction period, the degree of peroxidation of oils in all samples sped up and reached a maximum. After this maximum, the peroxides were further oxidized to secondary products such as aldehydes, ketones, and acids with off-flavours, thus resulting in the decline of absorbance (Figure 7). The kinetic pattern in our study was typical for edible oil peroxidation [58]. Table 2 shows that, compared to the control, all microcapsules had longer induction periods, and longer time to reach maximum peroxidation, and higher percent of inhibition of peroxidation. These results demonstrate the protecting effect of the coacervate shell against oil oxidation. The coacervate shell plays the role of a physical barrier to the diffusion of oxygen, heat, and light, hence retarding the oil oxidation [59].

For both drying methods, increasing the shell:core ratio resulted in higher stability (longer induction and peroxidation times) possibly due to the decrease in surface oil portion and increase in the shell thickness, which prevents the diffusion of oxygen into microcapsules.

For every shell:core ratio, the oil in FD microcapsules was oxidized slower than in OD microcapsules (higher percent of inhibition). This result is in accordance with another study, in which methyl linoleate encapsulated with gum arabic by FD showed higher oxidative stability than by OD [60]. However, this result seems contradictory to another study, in which oils in FD microcapsules are less stable

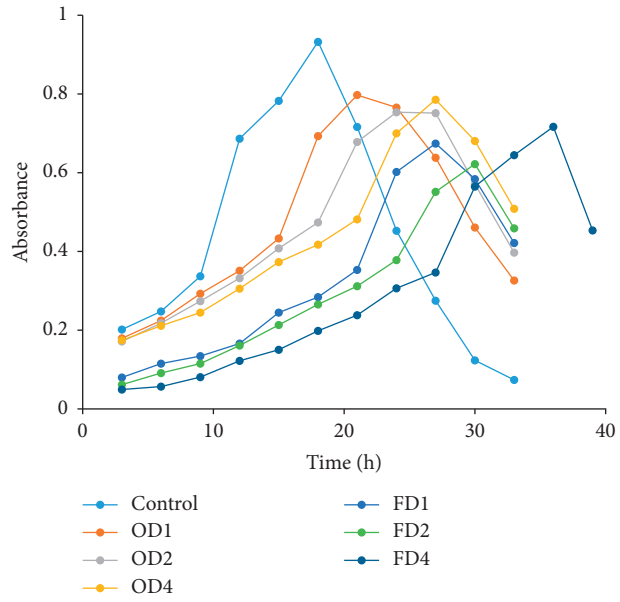

Figure 7: Kinetics of soybean oil peroxidation in OD and FD microcapsules with shell : core ratios of 1, 2, and 4.

than oils in spray-dried microcapsules due to the porous structure of FD microcapsules, which facilitates oxygen diffusion and oil oxidation [61]. The reason for this contradiction can be that the high temperature and long drying time during OD produced minor components, such as free fatty acids due to partial hydrolysis, and thermally oxidized compounds, which accelerated the autoxidation of soybean oil. These compounds contain both hydrophilic and hydrophobic groups and thus can act as an emulsifier, lower the surface tension, increase the introduction of oxygen into the oil, and accelerate the oil oxidation [62].

3.6. In Vitro Release of Curcumin in Simulated Gastric and Intestinal Fluids. The release of bioactive ingredients from coacervate microcapsules in the digestive tract is a complicated process and plays an important role in the uptake, distribution, and bioavailability of these bioactive components. In the gastric digestion, the simultaneous effects of enzymes and acidic pH 1-2 often destroy some of the bioactive compounds, normally in emulsions [63, 64]. A low release of encapsulated ingredients in the gastric fluid and a high release in the intestinal fluid are desired properties of microcapsules [65]. The percent of curcumin release at various conditions is shown in Table 3.

Freeze-drying (instead of oven-drying), using pepsin, adding SIF after SGF, and decreasing the shell:core ratio significantly ($p < 0.001$) increased curcumin release from the microcapsules. The enhancing effect of freeze-drying compared to oven-drying is due to the porous structure of microcapsules that facilitates their contact with acids and enzymes in the simulated fluids. In SGF, pepsin partially digests gelatin and acids partially hydrolyze HKG in the shell, thus releasing a part of curcumin in oil droplets. In SIF, pancreatin (a mixture of amylase, lipase, and protease) further digests gelatin in the shell and the oil droplet in the

Table 2: Kinetics characteristics of peroxidation of soybean oil in microcapsules with different drying methods and shell : core ratios.

	Control	OD1	OD2	OD4	FD1	FD2	FD4
Induction (h)	9	15	18	21	21	24	27
Maximum peroxidation (h)	18	21	24	27	27	30	36
Inhibition of peroxidation (%)	0	25.0 ± 0.4^{f}	49.2 ± 0.2^{e}	55.3 ± 0.2^{d}	69.5 ± 0.5^{c}	71.5 ± 0.9^{b}	78.9 ± 0.1^{a}

Results are expressed as mean ± standard deviation ($n = 3$). Means in the same row with different letters are significantly different based on Duncan multiple range test.

Table 3: Percent of curcumin released in different simulated digestive fluids.

	Without pepsin		With pepsin	
	SGF (%)	SGF + SIF (%)	SGF (%)	SGF + SIF (%)
OD1	10.5 ± 0.1^{c}	51.9 ± 0.1^{d}	26.3 ± 0.2^{d}	72.7 ± 0.4^{d}
OD2	9.9 ± 0.5^{b}	48.5 ± 0.3^{c}	21.7 ± 0.5^{c}	66.4 ± 0.1^{c}
OD4	8.9 ± 0.1^{a}	31.1 ± 0.5^{a}	15.0 ± 0.4^{a}	41.2 ± 0.7^{a}
FD1	24.6 ± 0.5^{f}	68.7 ± 0.6^{f}	31.1 ± 0.36^{f}	89.2 ± 0.3^{f}
FD2	20.1 ± 0.1^{e}	62.1 ± 0.2^{e}	27.6 ± 0.2^{e}	79.9 ± 0.4^{e}
FD4	12.1 ± 0.1^{d}	46.3 ± 0.9^{b}	18.0 ± 0.2^{b}	64.1 ± 0.6^{b}

Results are expressed as mean ± standard deviation ($n = 3$). Means in the same column with different letters are significantly different based on Duncan multiple range test.

core, thus facilitating the release of curcumin. It should be noted that HKG is a soluble dietary fibre and cannot be digested by the enzymes. Increasing the shell:core ratio thickens the shell, thus requiring more time for the enzymes to digest and resulting in a slower release of curcumin.

4. Conclusion

Hydrolyzed karaya gum forms complex coacervates with gelatin A with a maximum yield at pH of 3.75 and 1 : 1 mass ratio. The complex coacervate can microencapsulate, protect edible oils from oxidation, and controllably release active ingredients in simulated intestinal fluids. Combinations of HKG with other polycationic polymers can be further explored, such as other proteins and chitosan. Although natural KG is an approved food additive (E 416), HKG has not been tested for toxicity and cannot be used in foods and pharmaceuticals in the present and near future. However, based on their close structures with acetyl groups in KG replaced by hydrogen, we suggest that HKG has close or even higher safety and biocompatibility and biodegradability than natural KG. Therefore, HKG can have other promising applications in food and pharmaceutical industries, such as an emulsifier, thickener, stabilizer, and carrier for active ingredients.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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