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Effect of ultrasound homogenisation on the stability of curcumin microencapsulated by spray-drying

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Abstract

Microencapsulated curcumin (MEC) that has been by spray-dried has the potential to improve curcumin stability during storage. In the present work, curcumin was encapsulated using soy lecithin and gum Arabic, and different ultrasound energy inputs (UE) for emulsion homogenisation were applied before spray-drying. The microencapsulation yield (MY), microencapsulation efficiency (ME), solubility, powder morphology, and curcumin degradation in the accelerated test were determined. The UE at 70 kJ/kg caused a 2.2-fold increase in the ME of the powder as compared to the control sample. Furthermore, increasing UE from 70 to 175 kJ/kg led to a decrease in particle size, MY, and ME by 32, 15, and 8.9%, respectively. The stability of MEC under different pH conditions was in the order of pH 2 > 7 > 9. Furthermore, MEC showed an improvement in curcumin stability after 30 days of light exposure at 70°C. In general, a lower UE energy showed better performance in terms of curcumin protection and stable morphology of the MEC powder. However, higher UE energy could create smaller particles, and increase product solubility.

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Introduction

Curcumin is the most active compound in *Curcuma longa* (Zheng *et al*., 2019), and was first isolated by Vogel and Pelletier (1815). Curcumin has a great potential for improving human health since it has antimicrobial and anti-inflammatory properties (Peng *et al*., 2019). In addition, curcumin has been used to treat cancers, diabetes, obesity, and Alzheimer's disease (Priyadarsini, 2014; Li *et al*., 2019). Curcumin is also used as a popular colouring in various food products (Joshi *et al*., 2013). Nevertheless, curcumin's incorporation into commercial products is a challenge due to its poor stability (Priyadarsini, 2014; Li *et al*., 2019). Curcumin is quickly degraded in aqueous solutions under neutral or alkaline conditions (Niu *et al*., 2012). Curcumin molecules degrade into *trans*-6-(40-hydroxy-30-methoxyphenyl)-2,4-dioxo-5-hexanal, ferulic acid, feruloyl methane, and vanillin (Niu *et al*., 2012).

Numerous challenges are faced by curcumin formulators when incorporating curcumin into commercial foods and trying to maintain its bioactive functions (Zheng *et al*., 2019). Over the last several decades, considerable efforts to develop curcumin microencapsulation techniques to stabilise curcumin properties have been undertaken (Niu *et al*., 2012). However, limited efforts are directed to develop a wall-core system for curcumin protection. Gastrointestinal conditions could hydrolyse these components, and the core materials may be released (Paulo and Santos, 2017). A promising method for curcumin microencapsulation (ME) is spray-drying, which is a rapid, modern, and large-scale applicable process (Shishir and Chen, 2017).

Commercial curcumin applications could face numerous challenges when incorporating curcumin

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into functional foods, especially due to its low chemical stability and poor water solubility (Zheng *et al*., 2019). Curcumin is degraded under intense light, in high temperatures, and in high water activity (Schieffer, 2002). According to Niu *et al*. (2012), changes in the carrier microstructure could effectively control curcumin properties. Most of the previous studies concerning curcumin ME have used single wall material, and did not evaluate the stability of curcumin powder during an accelerated time period.

Wall material is the most critical factor affecting core stability (Shishir and Chen, 2017). According to Shishir and Chen (2017), the choice of wall material must be adaptable to the physico-chemical behaviours of the core. According to Le *et al*. (2017), a combination of protein and carbohydrate could enhance the protective capabilities of wall material over a single wall material. Common carbohydrate polymers used in wall materials include starches, maltodextrins, dextrins, and gum Arabic; while common protein polymers are whey protein, casein, and lecithin (Truong *et al*., 2005; Igual *et al*., 2014; Shishir and Chen, 2017). In the research of Nguyen *et al*. (2021), the combination of gum Arabic and lecithin produced good curcumin retention, and high powder solubility. According to Cano-Chauca *et al*. (2005), gum Arabic is a suitable wall material for sensitive components, and lecithin causes dispersion of curcumin, and a decrease in droplet collision before spray-drying (Nguyen *et al*., 2021).

Besides the selected wall material, the mean size of the emulsion droplet plays an essential role in emulsion stability (Jena and Das, 2006). According to Gharsallaoui *et al*. (2007), droplet size affects the powder's ME efficiency. Unsuitable homogenisation techniques could accelerate core degradation or lead to auto-oxidation of the sensitive components (Serfert *et al*., 2009). In a report by Serfert *et al*. (2009), it was found that intensive disturbance of an emulsion caused enhancement of oxygen diffusion and creation of free radicals, which led to an increase in the oxidation rate of the core. Ultrasound is a suitable technique for improving the emulsion's homogenised droplet size before spray-drying (Wu *et al*., 2000). Ultrasound homogenisation has positive effects on spray-drying, primarily due to energy saving, low processing costs (Silva *et al*., 2015), and an increase in ME efficiency, and stability of the emulsion (Soottitantawat *et al*., 2005). According to Alcântara *et al*. (2019), ultrasound could improve the efficiency of microencapsulation by up to 90%.

In the present work, the influence of ultrasound homogenisation at different energy inputs was examined. The accelerated conditions consisted of the temperature of 8°C (mimicking the temperature in a supermarket refrigerator) and 70°C (the accelerated storage temperature), light exposure (daily sunlight condition), and different pH levels (pH 2 - 9). The present work investigated the effects of ultrasound energy (UE) input on the stability of microencapsulated curcumin (MEC) under accelerated storage conditions. Changes in mean particle size, powder particle morphologies, microencapsulation yield (MY), microencapsulation efficiency (ME), solubility, and curcumin degradation in the accelerated test were observed.

Materials and methods

Materials

Soy lecithin, gum Arabic, and ethanol were purchased from Xilong Scientific (China), and used without further purification. Curcumin was synthesised from vanillin (Solvay, Belgium) and 2,4-pentanedione (Acros Organics, Belgium) using a procedure described elsewhere (Pabon, 1964), and purified three times *via* recrystallisation in 95% ethanol. All solvents and chemicals used for the extraction were of analytical grade (Merck Specialities Private Limited, Germany).

The low-hydrophilic-lipophiliic (HLB) emulsifiers, soybean lecithin (phospholipids ≥ 97.0%, lysophosphatidylcholine ≤ 10%), and gum Arabic were also purchased from Xilong Scientific (China).

Methods

Emulsion preparation

The ME procedure of curcumin with soy lecithin and gum Arabic was adapted from other studies (Jin *et al*., 2016; Bucurescu *et al*., 2018). A complex between curcumin and lecithin was produced by mixing and stirring Solution A (5 g of soy lecithin in 32 mL of butyl acetate) and Solution B (250 mg of curcumin in 80 mL of ethanol) for 15 min. The mixture was then homogenised (Ultra Turrax Homogenizer, IKA, Germany) at 15,000 rpm for 5 min. The amount of water in the emulsion was calculated to achieve an emulsion dry matter content of 12% w/w. The carrier content used was based on

the optimised procedure in our previous study (Nguyen *et al*., 2021).

Homogenisation

The mixture was then added drop-wise into an aqueous solution of gum Arabic under magnetic stirring and subsequent ultrasonic homogenisation (Ultrasonic Technology UP100H, Hielscher, Germany) with an ultrasonic probe of 18 mm diameter, 20 kHz frequency, and power of 100 W in a temperature-controlled thermostatic bath (10°C). The probe height of the ultrasound in contact with the emulsions was standardised at 40 mm. Calculation of ultrasound energy input was done using Eq. 1:

$$E_s = \frac{P.t}{TSS.V} \quad \text{(Eq. 1)}$$

According to Yan *et al*. (2010), the specific ultrasonic energy (E) was calculated using the ultrasonic power (P), multiplied by the ultrasonic time (t), and divided by the sample volume (V) and the initial total solids concentration (TSS). Based on the preliminary test, four different levels of ultrasound energy input (UE) were applied: (i) 75, (ii) 105, (iii) 140, and (iv) 175 kJ/kg.

Spray-drying

The emulsion was then spray-dried at 165°C (Mini Spray Dryer B290, Büchi) to obtain the MEC powder (Wang *et al*., 2009). The emulsions were produced with a 0.5 mm diameter spray nozzle under a spraying pressure of 2.5 bar, and hot air co-current downward flow. The inlet air temperature was adjusted to 165 ± 2°C, and the outlet temperature was maintained at 60 ± 5°C at an average feed rate of 7 mL/min.

Moisture content

After centrifugation, dry matter content was determined for all raw materials, including fresh and dried turmeric, and the resulting turmeric sediment. The dry matter measurements were performed according to Le-Tan *et al*. (2021). The dry matter content was used to calculate the total curcuminoid recovery based on a dry weight basis. Around 1 g of the turmeric was weighed in an aluminium pan, and dried at 103°C for 4 h. The dry matter was calculated using Eq. 2:

$$DM\ (\%) = \frac{(m_1 - m_p)}{m_0} \quad \text{(Eq. 2)}$$

where, DM: dry matter (%); m_p: weight (g) of the empty pan; m_0: sample weight (g) before drying; and m_1: weight (g) of the sample and the pan after drying.

Microencapsulation efficiency and yield

The ME and MY measurements were obtained according to Le *et al*. (2017). For microencapsulation efficiency (ME) measurement, the MEC powder (100 mg) was added to 40 mL of ethanol. The mixture was gently shaken for 1 min, and filtered with a nylon filter (450 µm pores). The filtrate was diluted properly, and the absorbance was measured at 424 nm to determine the MEC (m_1). To break the microcapsules, the residue was dried, dissolved in distilled water, and centrifuged at 10,000 rpm for 20 min. The solid curcumin was dissolved in ethanol, and quantified based on absorbance at 424 nm (m_2). The supernatant suspension containing curcumin and lecithin underwent extraction with butyl acetate. The upper layer was vacuum evaporated, and the resulting solid was dissolved in ethanol quantified as previously described (m_3). ME efficiency was calculated using Eq. 3:

$$ME\ (\%) = \frac{m_2 + m_3}{m_1 + m_2 + m_3} . 100 \quad \text{(Eq. 3)}$$

Using the ME yield (MY) measurement, MY was defined as a ratio of the mass of the total curcumin of the spray-dried powder to the mass of the total curcumin of the emulsion before spray-drying (Le *et al*., 2017).

Solubility

The spray-dried powder (m_0) was dissolved in 50 mL of distilled water with magnetic stirring for 5 min. The suspension was filtered, and the filtrate was then dried at 105°C until the mass (m_1) did not change (Zuanon *et al*., 2013). The solubility of the spray-dried powder was calculated using Eq. 4:

$$Solubility\ (\%) = \frac{m_1}{m_0} . 100 \quad \text{(Eq. 4)}$$

Particle size

The powders' particle size distributions (PSD) were measured using a laser diffraction system (LA-960, Horiba, Kyoto, Japan) equipped with liquid and dry dispersion units. The particle size of samples was measured in the liquid dispersion system filled with water (150 mL), and then ultrasonicated for 15 s, which was used to avoid particle agglomeration.

Agitation was continuously applied during the measurement. All evaluations were performed in triplicate.

Morphology

Morphologies and sizes of the spray-dried microcapsules were determined by field-emission scanning electron microscope ([FE-SEM], S4800, Hitachi, Japan). The analysed samples were adhered to metallic supports (stubs) using double-sided metallic tape, and observed under SEM for image acquisition.

Curcumin quantification

The curcumin content was determined by spectrophotometry. Sediment separation was accelerated by centrifugation of the dispersion at 4,000 rpm for 10 min. The supernatant was diluted ten times with ethanol (96%). The absorbance at wavelength 424 nm was monitored in an ultraviolet/visible (UV/vis) spectrophotometer (UV-1800, Shimadzu, Japan). A previously prepared calibration curve (n = 6; R^2 = 0.9969) of curcumin absorbance in ethanol was used to calculate the curcumin content in µg/mL using Eq. 5:

$$\text{TC (µg/ mL)} = \frac{(A_{424nm} - 0.0216) \,.\text{Dilution Factor}}{0.01384 \,.\text{Sample Weight}} \quad \text{(Eq. 5)}$$

Curcumin retention in storage test

To evaluate the effect of microencapsulation with gum Arabic on curcumin stability, the spray-dried powder with a known amount of curcumin (m_0) was suspended in water, and then subjected to different stability testing conditions. An aliquot of the suspension was taken at regular time points to quantify the amount of remaining curcumin (m_1) based on a standard curcumin curve. The control samples for the stability tests consisted of curcumin suspensions in water with Tween 80 as an emulsifier. The relative stability of MEC was calculated using Eq. 6:

$$\text{Curcumin retention (\%)} = \frac{m_1}{m_0} \,.\, 100 \quad \text{(Eq. 6)}$$

The MEC stability was tested under the influence of different pH values (from pH 2 to 6 in acetate buffer, and pH 7 to 9 in phosphate buffer), light (in the dark or under 400 lumen/m^2 irradiation), and temperature (8°C in a refrigerator, or 70°C in a drying oven).

Statistical analyses

All tests and analyses were conducted in triplicates. Statistical analyses were performed using Statgraphics Centurion XVII, version 17.1.04 (Statpoint Technologies, Inc., Warrenton, VA, USA). Results were expressed as mean ± standard deviations (SD) of three single determinations (One-way analysis of variance (ANOVA) with $\alpha = 0.05$), and Fisher's least significance test was used to establish the significance of differences among the mean values.

Results and discussion

Effect of ultrasound energy input on physical properties of microencapsulation curcumin powder

Microencapsulation efficiency

It is notable that with the increase in UE, the ME decreased (Figure 1a). When the UE increased from 70 to 105 kJ/kg, the ME decreased by 3.4%. When the energy input increased to 175 kJ/kg, the ME decreased by 8.9% (Figure 1a). It can also be seen that the surface curcumin increase corresponded to an increase in the ultrasound input energy (Table 1). Notably, the mean size of the emulsion droplet decreased with higher energy input (Table 1). The reason for these observations could be based on two hypotheses: (i) the loss of small particles in exhaust air (decreasing MY), and (ii) the high amount of surface curcumin (decreasing ME). According to Soottitantawat *et al*. (2005), ultrasound treatment could lead to a significant decrease in the droplet size. In a report by Alcântara *et al*. (2019), the authors agreed with the finding that a decrease in droplet size occurred with a corresponding increase in homogenisation energy input.

The larger particle size caused an increase in ME (Jafari *et al*., 2008). It could be shown in the present work that when particles size decreased by 32%, the surface area of the powder increased by 28.4% (Table 1). Numerous previous studies have highlighted the role of span value in the efficiency of spray-drying (Jafari *et al*., 2007; Koç *et al*., 2015). A high span value results in the spread of particle size distribution, and low uniformity sizes. The results revealed that when the UE increased from 70 to 175

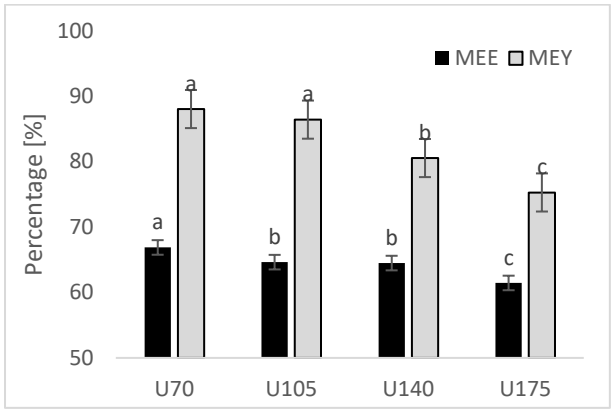
kJ/kg, the span value increased by 84.5% (Table 1). This phenomenon could have affected the morphology of the powder, thus resulting in low ME.

Microencapsulation yield

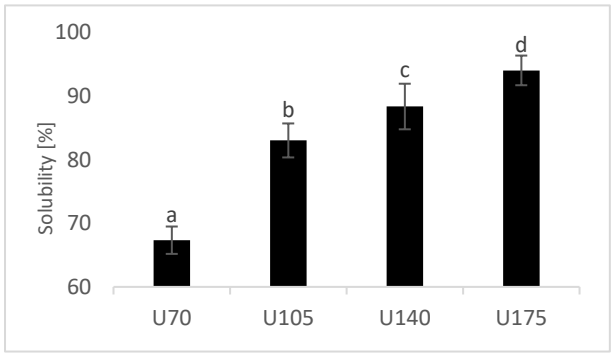
As can be seen from Table 1 and Figure 2, an increase in the ultrasonic energy led to a decrease in particle size. Regarding small particle size, in cases in which heat transfer and drying velocity are rapid, small particles may have been lost in the exhaust air. It can be seen from Figure 1b that the MY decreased with higher UE. This agreed with a previous study, in which it was described that small particles tend to stick on the cyclone wall, primarily caused by electrostatic interactions (Keshani *et al*., 2015). Besides, fine particle size leads to the powder's lower flowability properties (Koç *et al*., 2015).

Solubility of microcapsule powder

It can be seen from Table 1 that when the mean particle size decreased by 31.2%, the total surface area and solubility of the curcumin powder increased by 65.1 and 39.6%, respectively. This confirmed the role of particle size in the solubility of the spray-dried powder. A smaller particle size could have caused an increase in the solubility of the curcumin powder due to its high surface area (de Barros Fernandes *et al*., 2016).



(a)



(b)

Figure 1. Effect of ultrasound energy inputs on **(a)** microencapsulation efficiency (%) and microencapsulation yield (%), and **(b)** solubility (%).

Table 1. Characteristics of microencapsulation powder at different ultrasound energy inputs.

	Moisture of powder (%)	**Mean size (μm)**	**Span**	**Surface area (cm^2/cm^3)**	**Surface curcumin (%)**
U70	2.41 ± 0.76^a	42.39 ± 1.16^a	4.25 ± 0.24^a	5543.80 ± 139.31^a	33.12 ± 1.05^a
U105	2.75 ± 0.56^b	40.95 ± 1.56^a	4.56 ± 0.17^a	5823.80 ± 67.03^b	37.37 ± 0.79^b
U140	2.93 ± 0.96^c	31.50 ± 1.96^b	5.97 ± 0.14^b	7673.00 ± 155.82^c	37.01 ± 1.9^b
U175	2.28 ± 0.42^d	29.18 ± 1.52^c	7.85 ± 0.12^c	9154.00 ± 127.92^d	42.55 ± 1.49^c

Means followed by different lower superscripts in the same column are significantly different ($p < 0.05$).

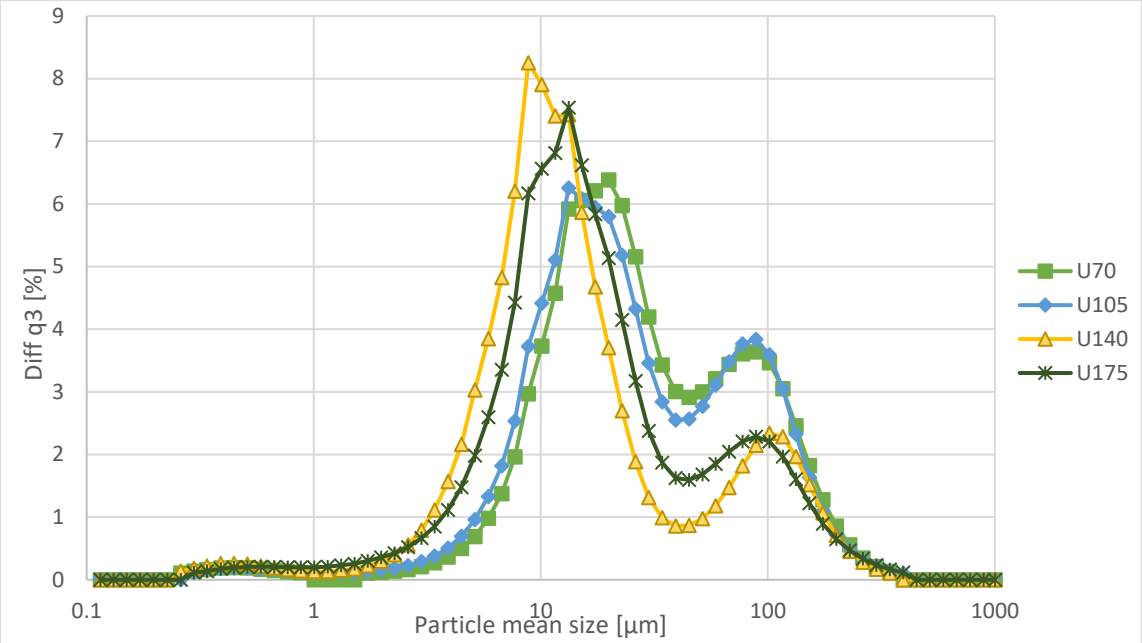
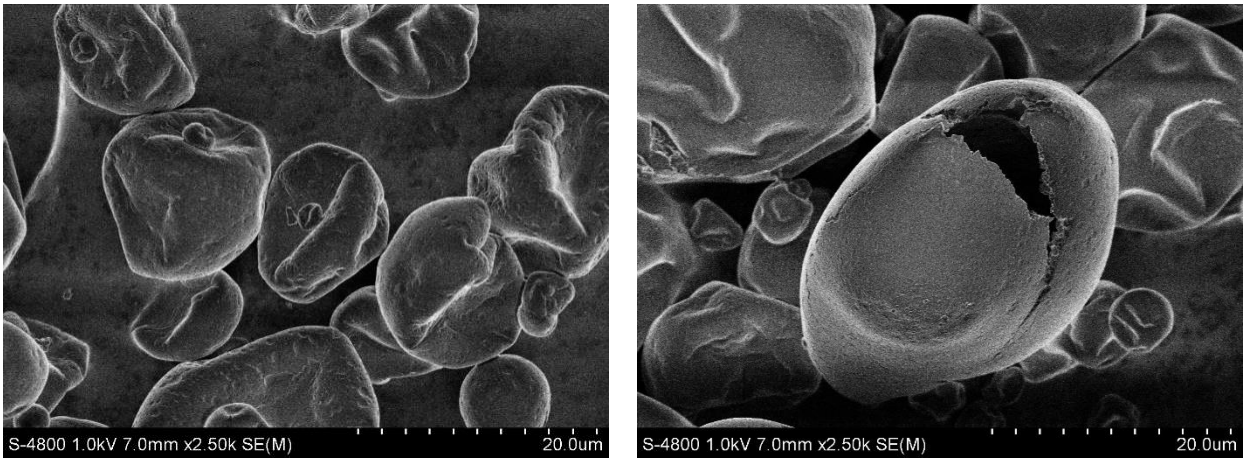


Figure 2. Differences in powder mean size of curcumin powder under different ultrasound treatments.

Effect of ultrasound energy input on stability of MEC powder

Curcumin degrades under exposure to light, high temperatures, and oxygen, thus limiting curcumin applications in food products (Suresh *et al.*, 2007; Osorio-Tobón *et al.*, 2014). ME is the convenient solution for protecting bioactive compounds and increasing their shelf life (Calvo *et al.*, 2012). Besides, MEC causes expansion of the curcumin applications in functional foods for commercial use. Interestingly, an effective homogenisation process could affect particle morphology leading to less shrinkage on the particle surface (Figure 3). The same phenomenon is shown in Figure 2; with the U70 treatment, the particle size became more evenly distributed.



(a) **(b)**

Figure 3. Scanning electron microscopy (SEM) images of powder particles under different ultrasound energy inputs: **(a)** U70, and **(b)** U175.

Stability of MEC powder under different pH conditions

A pH higher than 9 was not evaluated because most food products are not found in highly alkaline conditions. The present work found that curcumin stability followed the order of pH 2 > 7 > 9. This agreed with previously reported studies in which curcumin was reported to be more stable under acidic pH conditions but unstable in alkaline conditions (Lestari and Indrayanto, 2014). According to Lestari and Indrayanto (2014), the primary degradation products of curcumin under alkaline conditions were vanillic acid and feruloylmethane.

In the present work, using the non-microencapsulated curcumin solution, the curcumin content decreased to 83% after 10 days. Besides, after 40 days, the remaining curcumin content was lower than 3% at pH 9 (Figure 4e). Under acidic conditions, the non-microencapsulated curcumin content remained at 79% after 40 days. This confirmed the role of pH in the stability of curcumin solution. Kharat *et al*. (2017) reported that more than 85% of curcumin was retained in the emulsion under acidic conditions, while lower than 53% of curcumin remained in the emulsion at pH 8.0 after incubation at 37°C for one month.

In the case of MEC, the reconstituted curcumin solution after 40 days remained 86.32, 79.55, and 75.06% of curcumin at pH values of 2, 7, and 9, respectively (Figure 4a). In a report by Zheng *et al.* (2019), the curcumin emulsion with soy protein maintained the creamy yellow colour after 36 days. In agreement with that finding, Kharat *et al*. (2017) showed the improvement of water dispersibility and chemical stability in the oil-in-water curcumin emulsion.

When compared with the control curcumin solution (Figure 4e), ME led to a significant improvement in curcumin retention in the solution (Figures 4a - 4d). A reason for this finding could be that at a low pH value, curcumin exists in a crystal form, which could be more stable than the dissolved form (Le-Tan *et al*., 2021). On the other hand, under alkaline pH conditions, curcumin dissolves in solution, and quickly degrades to vanillic acid and feruloylmethane (Lestari and Indrayanto, 2014). In a recent report by Nguyen *et al*. (2021), the authors concluded that the -COOH group in the gum Arabic polymeric chain could neutralise the hydroxyl ions (OH^-) in neutral and alkaline solutions.

It was found that curcumin retention decreased when UE increased. With the increase in UE from 70 to 175 kJ/kg, the remaining curcumin slightly decreased by 17% at pH 9 after 40 days (Figures 4a and 4d). The results established that appropriate homogenisation could cause stabilisation of the suspension, and an improvement in the core stability during the storage time.

Stability of MEC powder under light exposure

In the report of Prathapan *et al*. (2009), the author concluded that curcumin was more stable in the dry form after photo-oxidation. MEC powder with moisture lower than 3% (Table 1) could play a vital role in improving the shelf life of curcumin. However, in the case of the reconstituted powder, the choice of carrier and optimised homogenisation could affect the photo-oxidative stability of curcumin.

Exposure of curcumin to light produces degradation products (Jankun *et al*., 2016), and the primary products were found to be vanillic acid and feruloylmethane (Lestari and Indrayanto, 2014). The current experimental setup aimed to gain further insight into the effect of light exposure on MEC stability.

Curcumin retention after 30 days of exposure to light is shown in Figure 5. In general, light exposure led to considerable degradation of curcumin during storage. As compared to the curcumin powder under dark conditions, the remaining curcumin in light-exposed samples decreased by 30 to 110% after 30 days (Figure 5).

In the control sample, curcumin retention significantly decreased in the first week (Figure 5). After three days, free curcumin in the control sample decreased by 60%; however, MEC decreased by only 6%. It was notable that after 12 days, 100% of the free curcumin was degraded while the MEC remained steady at 42%. In a report by Schieffer (2002), a control sample stored in one day of sunlight could lose up to 80% of its curcumin content. In addition, Kiamahalleh *et al*. (2016) indicated considerable curcumin degradation occurred in the presence of light. Nevertheless, MEC content remained fairly constant after 30 days of light exposure (Figure 5). This indicated the role of emulsion in curcumin protection, and the addition of emulsifiers to curcumin solution could lead to a decrease in curcumin degradation (Lestari and Indrayanto, 2014).

In general, the U70 sample caused less curcumin decrease than other samples (Figure 5). After six days of light exposure, the remaining curcumin in the U70 sample was 66%, while 40, 45, and 17% remained in U105, U140, and U175, respectively. The reason for this observation could be that U70 had a more uniform particle size, larger particles, and lower surface area (Table 1). In addition, the morphology of the powder surface showed good result with less shrinkage and powder collapse (Figure 3). According to Alcântara *et al*. (2019), suitable ultrasound homogenisation could lead to an improvement in the characteristics of the particles and the ME efficiency.

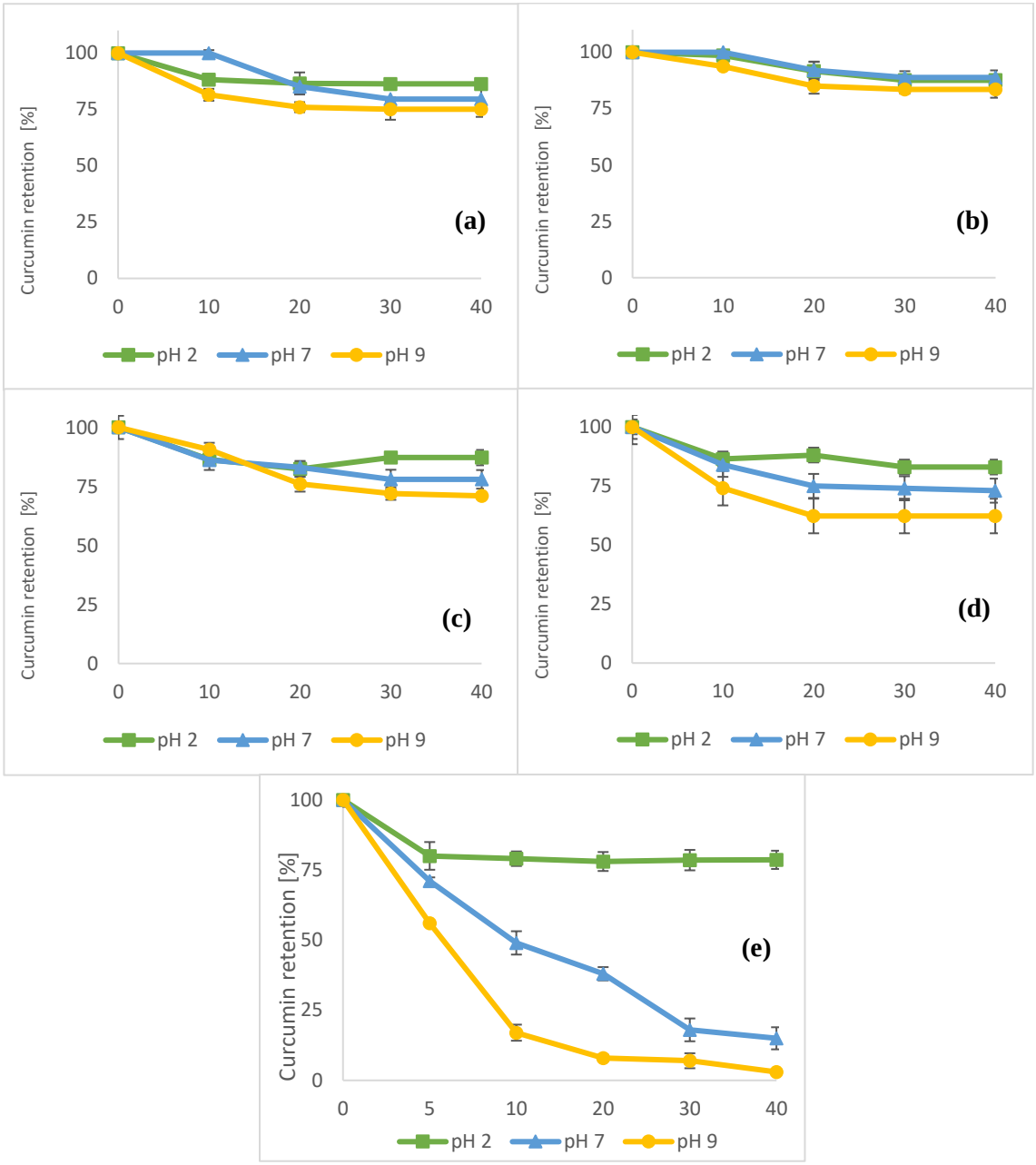


Figure 4. Effect of pH condition on curcumin retention in reconstituted microcapsule powder. **(a)** U70, **(b)** U105, **(c)** U140, **(d)** U175, and **(e)** control sample.

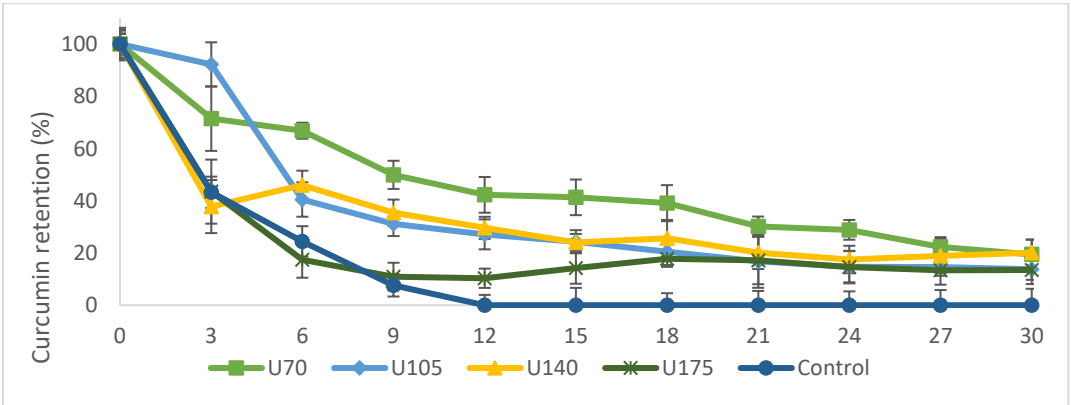


Figure 5. Effect of light exposure on curcumin retention in reconstituted microcapsule powder.

Stability of MEC powder under different temperatures

In our experimental setup, the MEC powder was exposed to temperature of 8 and 70°C over 30 days to monitor curcumin retention. Numerous processes in the food industry are related to thermal processing. A thermal stability experiment is vital for observing MEC stability under different temperature conditions. The 8°C condition mimicked supermarket temperatures, while 70°C is the accelerated condition for curcumin degradation. Curcumin content in the aqueous solution is shown in Figure 5. The stability of MEC improved significantly after 30 days as compared to free curcumin. After nine days, the curcumin remaining in the control sample at 70°C was 8%, while 60% of MEC remained under the same conditions (Figure 5).

Furthermore, the appropriate homogenising energy input (U70) led to enhancement of the stability of MEC by 80% in comparison with high energy input conditions (U175). According to Alcântara *et al*. (2019), an optimised homogenisation process could thoroughly disperse the core and wall materials, and increase the process's efficiency. Furthermore a possible hypothesis for this observation was that the uniform particle of the U70 sample decreased the phase separation during the storage period (Table 1). In the present work, increasing ultrasound input energy affected homogenised droplet size, and led to the decrease in curcumin stability in the water phase (Figure 5). This confirmed the role of uniform particle sizes and particle morphologies in terms of MEC stability.

Conclusion

Curcumin ME *via* spray-drying was a suitable technique for enhancing curcumin stability and its application into commercial food products. The combination of soy lecithin and gum Arabic with low UE input for homogenisation was the optimal condition for creating MEC powder. The ME increased by 220% as compared to the untreated sample. In the present experimental setup at a UE level of 70 kJ/kg, the MEC powder presented suitable morphology particles with less shrinkage. In addition, increasing UE from 70 to 175 kJ/kg led to a decrease in particle size, MY, and ME by 32, 15, and 8.9%, respectively. However, the low UE treatment could cause a decrease in the solubility of the powder, and an increase in particle size. In general, the U70 treatment condition yielded high ME and MY, and the better stability for the resulting powder under accelerated conditions with different pH values, temperatures, and levels of light exposure.

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