การกักเก็บเคอร์คูมินโดยการอบแห้งแบบพ่นฝอยโดยใช้ Tween 80 ร่วมกับไคโตซาน
Encapsulation of Curcumin by Spray Drying Using the Combination of Tween 80 and Chitosan

อวานี ฮามัด[1, 3] สาริศา สุริยรักษ์[1, 2] ชาลีดา บรมพิชัยชาติกุล[1, 2*]
Alwani Hamad[1, 3], Sarisa Suriyarak[1,2], Chaleeda Boromphichaichartkul[1,2*]

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บทคัดย่อ

ในงานวิจัยนี้ศึกษาการใช้ Tween 80 ร่วมกับไคโตซาน (อัตราส่วน 1/0, 1/1, 1/2, 1/3 และ 0/1 %w/w) ในการกักเก็บสารเคอร์คูมินในรูปไมโครแคปซูลโดยการอบแห้งแบบพ่นฝอย ในการทดลองวิเคราะห์ค่าความหนืด ปริมาณของแข็งทั้งหมดของสารอิมัลชันที่ป้อนเข้า ปริมาณสารที่ได้สุทธิจากกระบวนการ และสมบัติทางเคมีกายภาพ (ค่ากิจกรรมของน้ำ ความชื้น ค่าการละลาย สี ความเข้มข้นของเคอร์คูมิน ประสิทธิภาพในการกักเก็บ และรูปถ่ายโครงสร้างของผงไมโครแคปซูล) รวมถึงค่าการต้านการเกิดออกซิเดชัน (DPPH และ FRAP) จากการทดลองพบว่าค่าความหนืดของสารอิมัลชันเคอร์คูมินที่ป้อนเข้าเพิ่มขึ้น (43.30-78.65 cP) เมื่อเพิ่มความเข้มข้นของไคโตซาน การใช้ Tween 80 ร่วมกับไคโตซานสามารถเพิ่มประสิทธิภาพการกักเก็บเคอร์คูมินในไมโครแคปซูลได้ แต่การเพิ่มความเข้มข้นของไคโตซาน กลับพบว่าลดปริมาณสารที่ได้สุทธิจากกระบวนการ (ร้อยละ 41.17-33.02) แต่ไม่มีนัยสำคัญ ฤทธิ์การต้านอนุมูลอิสระในการที่วัดด้วยวิธี DPPH มีแนวโน้มลดลง (ประมาณร้อยละ 15) แต่ FRAP และค่าสี *(L*, a** และ *b*)* ไม่แตกต่างอย่างมีนัยสำคัญ อีกทั้งพบรอยย่นบนผิวของไมโครแคปซูลของเคอร์คูมินเมื่อมองภายใต้กล้องจุลทรรศ์อิเล็กตรอน ในกรณีของตัวอย่างที่ไม่มีการเติม Tween 80 พบว่าตัวอย่างผงมีการเกาะกันเป็นก้อนไม่มีรูปทรงแน่นอน โดยสรุปผงไมโครแคปซูลที่ได้จากการใช้ไคโตซานร่วมกับ Tween 80 ที่ อัตราส่วน 1:1 โดยน้ำหนัก มีประสิทธิภาพในการกักเก็บสูงที่สุด (ร้อยละ 57.48) รวมทั้งมีฤทธิ์การต้านการเกิดออกซิเดชัน ที่วัดโดยวิธี DPPH และ FRAP สูงสุด (49.82, 17.12 mM TE/g ตามลำดับ) ดังนั้นการใช้ Tween 80 ร่วมกับ ไคโตซาน เป็นสารเคลือบส่งผลเชิงบวกต่อสมบัติการกักเก็บเคอร์คูมินในไมโครแคปซูล

คำสำคัญ: เคอร์คูมิน กระบวนการกักเก็บ การอบแห้งแบบพ่นฝอย ไคโตซาน ฤทธิ์การต้านออกซิเดชัน

ABSTRACT

This study investigated the effect of the combination of Tween 80 and Chitosan (1/0, 1/1, 1/2, 1/3, and 0/1 (%w/w)) as encapsulated agents in the encapsulation of curcumin using spray drying. Viscosity, solid content of feed emulsion, process yield, physicochemical properties (water activity, moisture content, solubility, color, curcumin concentration, encapsulation efficiency (EE) and microstructure by SEM) and antioxidant activity of the encapsulated powder were analyzed. It was found that the viscosity of curcumin feed emulsion was increased (43.30-78.65 cP) with the elevation of chitosan concentration. The presence of Tween 80 and chitosan improved the encapsulation efficiency of curcumin in microcapsule. However, the increasing level of chitosan reduced process yield (41.17-33.02%) and antioxidant activity in term of DPPH (about 15%) but FRAP and color parameter *(L*, a* and b*)* are not significantly different as well as it increased the wrinkled microstructure of powders. Without Tween 80, most powders were in a clump, agglomerated and irregular shape. The encapsulated powder sample with a combination of Tween 80 and chitosan at the ratio of 1:1(%w/w) demonstrated the highest EE (57.43%) and antioxidant activity by DPPH and FRAP method (49.82, 17.12 mM TE/g, respectively). Overall, the combination of Tween 80 and chitosan as encapsulation agents showed increasing desirable properties of encapsulation efficiency of curcumin in the microcapsule.

Keywords: curcumin, encapsulation, spray drying, chitosan, antioxidant activity

* Corresponding author e-mail: chaleedab@hotmail.com, chaleeda.b@chula.ac.th
[1]Department of Food Technology, Faculty of Science, Chulalongkorn University
[2]Emerging Process for Food Functionality design (EPFFD) research unit, Department of Food Technology, Faculty of Science, Chulalongkorn University
[3]Department of Chemical Engineering, Faculty of Engineering and Science, Universitas Muhammadiyah Purwokerto, Indonesia

INTRODUCTION

Curcumin is a polyphenol that contributes to many health benefits. It has already been confirmed that medicinal uses of curcumin were attributed to its antioxidant, anticancer, and anti-inflammation activities [1]. The essential biological activities of curcumin are supported by the incorporation of this compound into food systems as functional food. However, curcumin physicochemical properties exhibit poor solubility and high rate of metabolic degradation. These drawback limited its applications into functional food [2].

Encapsulation is one of the promising techniques for delivering and improving the stability and solubility of curcumin. Spray drying is an affordable alternative technique to encapsulate curcumin. Spray-dried curcumins have been produced with many encapsulation agents or wall materials, but their limitation of physical and chemical properties still need to be improved**.** Maltodextrin is a wall material which is common carrier material but less film-forming ability which is important for drying efficiency [3]. Although many encapsulating agents can be applied, both individually and in combination, some properties should be investigated for a better productivity. The amount and stability of curcumin encapsulated in microcapsules is still unsatisfied by manufacturers. Therefore, improving the formulation and development process for encapsulating curcumin via spray drying is needed to obtain a better stability, process yield and encapsulation efficiency of curcumin microcapsules. The incorporation of curcumin with biopolymer coating is another method to improve curcumin stability [4].

Chitosan is a cationic biopolymer with excellent compatible and biodegradable properties. It has been reported that chitosan coating at multilayer curcumin loaded emulsions resulted in better stability, and it can inhibit degradation of curcumin during digestion [5]. The curcumin encapsulated in sub-micrometer of Tween 20/Chitosan particles for drug delivery also exhibit spherical shape particles as well as improvement of encapsulation efficiency [6]. Tween 80 (polyoxyethylene sorbitan monooleate) has a longer aliphatic tail and therefore more lipophilic than Tween 20 (polyoxyethylene sorbitan monolaurate). The application of Tween 80 to stabilize curcumin encapsulation in emulsion has more benefits toward the use of Tween 20 due to the long tail of Tween 80 which contributed in the pronounced of steric hindrance effects [5, 7, 8]. Hence, the combination of chitosan and Tween 80 in the formulation of curcumin emulsion containing maltodextrin may improve the efficiency of the encapsulated curcumin powder by spray drying. However, the study of using Tween 80 combined with chitosan is limited. Thus, this study was aimed to encapsulate curcumin in form of oil-in-water emulsion then converted to powder by using spray drying. Encapsulation efficiency is aimed to improve by studying the effects of the combination of Tween 80 and chitosan on physical and chemical properties as well as antioxidant activity of encapsulated curcumin powder.

***** Corresponding author e-mail: chaleedab@hotmail.com, chaleeda.b@chula.ac.th
[1]Department of Food Technology, Faculty of Science, Chulalongkorn University
[2]Emerging Process for Food Functionality design (EPFFD) research unit, Department of Food Technology, Faculty of Science, Chulalongkorn University
[3]Department of Chemical Engineering, Faculty of Engineering and Science, Universitas Muhammadiyah Purwokerto, Indonesia

MATERIALS AND METHODS

1. Materials

Curcumin (contained >95% curcuminoids purity, Vitajoy Biotech Co.Ltd, Suzhou, China), Medium-chain triglyceride (MCT) oil (Vicchi Enterprise Co.Ltd, Bangkok Thailand), Chitosan (food grade) with degree of deacetylation >90%; MW 35 kDa, Maltodextrin with dextrose equivalent (DE) value 10-15 (Bronson & Jacobs international Co. Ltd Shanghai, China), 2,2-diphenyl-1-picrylhydrazyl (DPPH reagent), gallic acid, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), and 2,4,6-tripyridyl-s-triazine (TPTZ).

2. Preparation of curcumin feed emulsion

The preparation of curcumin emulsion was followed the method by Kharat et al. (2017) with some modifications [9]. The preparation of the oil phase was done by mixing curcumin into MCT oils at the concentration of 17.01 mg/ml at 80°C for 10 minutes. An aqueous phase was prepared by mixing surfactant (Tween80) in phosphate buffer (10 mM, pH 6) at room temperature for at least 1h at 300 rpm by a mechanical overhead stirrer. The 2.5%w/w chitosan (30 kDa) that was dissolved into 1 %v/v acetic acid then was added to the aqueous phase. The ratio of combination of Tween 80 and chitosan were 1/0, 1/2, 1/3 and 0/1, %w/w. Maltodextrin with DE 10-15 (20%, w/w) was mixed as wall material into aqueous phases and then stirred until it was fully dissolved. A stock emulsion, 10%w/w O/W emulsion was prepared by mixed the oil and aqueous phases together by stirring for 30 minutes and then following by mixing using an Ultra Turrax® T18 Homogenizer for 5 minutes. The final emulsion sample was further used in spray drying process straight away.

The viscosity of the emulsion feed sample was investigated following the methods by Dantas et al. (2018) [10], and the solid content follows methods according to the Association of Official Analytical Chemists (AOAC) [11].

3. Spray drying

The spray drying process was conducted to a mini-spray dryer (BÜCHI B-290, Flawil, Switzerland) with a standard 0.5-mm nozzle. The curcumin feed emulsions (21.71- 23.89%) were spray-dried under the inlet temperature of 175°C and solution flow rate at 4 ml/min. The outlet temperature was around 90±5°C, and curcumin powder was collected and kept in a desiccator and prevented from the light by covering with aluminum foil.

4. Process yield and physical characterization of encapsulates powders

The process yield was determined by the ratio of the mass of powders collected to the total mass of solids in the feed. The % process yield was calculated according to Equation (1) [12].

$$\%\,Process\ yield = \frac{Powder\ obtained\ at\ the\ spray\ dryer\ x\ 100\%}{Total\ solid\ content\ in\ sample\ volume} \quad (1)$$

The analysis of water activity and moisture content of curcumin encapsulated powder was conducted by following method by Dantas et al. (2018) [10] and the AOAC method, respectively. In addition, the microstructure of curcumin microcapsule was examined by

* Corresponding author e-mail: chaleedab@hotmail.com, chaleeda.b@chula.ac.th
[1]ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
[2]หน่วยปฏิบัติการวิจัยกระบวนการผลิตเพื่อออกแบบสมบัติเชิงหน้าที่ของอาหาร ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
[3]ภาควิชาวิศวกรรมเคมี คณะวิศวกรรมศาสตร์และวิทยาศาสตร์ มหาวิทยาลัยมูฮัมหมัดดียา พูโวกาโต อินโดนีเซีย

Scanning Electron Microscope (SEM) at 5 kV acceleration voltage type JSM-5800LV, JEOL. The solubility of encapsulated curcumin powders represented as water solubility index (WSI) was calculated as follows the method conducted by Ahmed et al. (2016)[13].

The color of the encapsulated curcumin powder was determined with the CIELAB color space (*L**, *a** and *b**) using a Minolta Chroma Meter CR-400 colorimeter (Konika Minolta, Osaka, Japan). The *L** value considers as sample luminosity, the *a** value indicates the color ranging from green to red, and the *b** shows the color varying from yellow to blue.

5. Encapsulation efficiency and curcumin concentration

The concentration of the curcumin was determined by UV-VIS spectroscopy method. Six mg of curcumin was dissolved in 100 ml of ethanol. Subsequently, 0.25, 0.5, 1, 2, 3 and 4 ml of solution were taken and diluted to 15 ml with alcohol, respectively, to obtain final curcumin concentrations of 1-6 mg. The absorption was observed at the maximum wavelength (λ max) of 425 nm. Absorbance as Y-axis was plotted versus curcumin concentration on x-axis to produce the standard curve of curcumin with equation of Y = 0.1325 X + 0.0878, R^2 = 0.9968 [9].

Analysis of total curcumin content (TCC) and surface curcumin content (SCC) were followed from methods from Laokuldilok, *et al.* (2015) with some modifications [14]. TCC determined as the internal and surface curcumin content of the encapsulated powders. SCC is defined as the non-encapsulated curcumin at the surface of the powder. The measurement of TCC and SCC using the spectroscopy method was conducted as previously described. The percentage of encapsulation efficiency (EE) was calculated according to Equation (2).

$$\% EE = \frac{(TCC - SCC)\left(\frac{mg}{g}\right) x\ 100\%}{TCC\left(\frac{mg}{g}\right)} \quad (2)$$

6. Evaluation of antioxidant activity (AA)

Two assays: DPPH and FRAP determined the antioxidant capacity of encapsulated curcumin powder. The DPPH radical scavenging capacity was investigated using the method as follows by Brand-Williams et al. (1995)[15]. The measurement using a spectrophotometer at absorbance of 517 nm was measured after the reaction for 30 min. The FRAP assay was conducted following the method reported by Thaipong et al. (2006) at an absorbance of 594 nm [16]. The AA was showed in mM Trolox Equivalent (TE) /g to dry based on powders.

7. Data Analysis

The results obtained on all data considered as mean ± standard deviation. Analysis of variance (ANOVA) was conducted to decide a significant difference ($p \leq 0.05$) among the samples, using the software SPSS 17.0 for the window (SPSS Inc., Chicago, IL. USA). Differences between means were expressed using Duncan's test. All measurements are performed in triplicate.

* Corresponding author e-mail: chaleedab@hotmail.com, chaleeda.b@chula.ac.th
[1]ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
[2]หน่วยปฏิบัติการวิจัยกระบวนการผลิตเพื่อออกแบบสมบัติเชิงหน้าที่ของอาหาร ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
[3]ภาควิชาวิศวกรรมเคมี คณะวิศวกรรมศาสตร์และวิทยาศาสตร์ มหาวิทยาลัยมูฮัมหมัดดียา พูโวกาโต อินโดนีเซีย

RESULTS AND DISCUSSION

1. Physical properties of feed emulsion

Emulsion physical properties evaluated in this study were viscosity and solid content parameters (Table 1). The added of chitosan into curcumin emulsion at 1-3%w/w did not affect solid content (p>0.05). However, increasing the concentration of chitosan from 1 to 3%w/w increased the viscosity of the final curcumin emulsion significantly (p<0.05).

Table 1 Physical properties of curcumin feed emulsion

Tween 80/ Chitosan ratio (%w/w)	Viscosity (mPa.s)	% Solid content
1.0/0.0	$43.30±0.28^{c}$	$21.71±4.98^{a}$
1.0/1.0	$44.87±0.31^{c}$	$23.29±1.09^{a}$
1.0/2.0	$67.30±1.13^{b}$	$23.37±2.19^{a}$
1.0/3.0	$78.65±2.89^{a}$	$23.28±3.66^{a}$
0.0/1.0	$46.30±6.36^{c}$	$23.80±1.06^{a}$

Different letters on superscript within a column were indicated that the values with significant differences (p < .05)

Chitosan is a biodegradable polymer produced from alkaline deacetylation processing of chitin. In industrial processing, chitin is extracted from crustaceans by acid treatment to dissolve calcium carbonate followed by alkaline extraction and decolorization. By partial deacetylation under alkaline condition, chitosan is obtained [17]. It is a cationic polyelectrolyte that formulated by primarily of (1⟶4)-linked β-*D*-glucosamine units and combined with *N*-acetyl-β-*D*-glucosamine units [17]. Many parameters influence the viscosity of chitosan solution such as its concentration and deacetylation degree. In general, for chitosan in solution and polyelectrolytes, the increasing of its concentration leads to the expansion of chains of chitosan polymer because of the electrostatic repulsions, which eventually increase the intrinsic viscosity [18]. The application of chitosan was usually combined with an emulsifier for encapsulation of bioactive compounds in the emulsion system. The use of chitosan alone cannot produce a stable emulsion [19-20]. It contributed as stabilizing via viscous electro-steric effect [21]. The increase of the chitosan concentration increases the intrinsic viscosity of the aqueous phase that resulted in restricted the droplet movement, eventually increases emulsion stability and encapsulation efficiency in emulsion when combining with emulsifiers [5, 22].

2. Characterization of the encapsulated curcumin powder

Process yield and physical properties of spray-dried encapsulated curcumin powders are presented in Table 2. In general, the process yield obtained is quite low i.e. <50% this may due to using mini spray dyer BUCHI B-290 that may limited the yield. The process yield of the curcumin powders was also affected by the presence of the emulsifier (Tween 80). In the formulation without Tween 80, the process yield was lower than other samples (p<0.05).

* Corresponding author e-mail: chaleedab@hotmail.com, chaleeda.b@chula.ac.th
[1]ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
[2]หน่วยปฏิบัติการวิจัยกระบวนการผลิตเพื่อออกแบบสมบัติเชิงหน้าที่ของอาหาร ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
[3]ภาควิชาวิศวกรรมเคมี คณะวิศวกรรมศาสตร์และวิทยาศาสตร์ มหาวิทยาลัยมูฮัมหมัดดียา พูโวกาโต อินโดนีเซีย

During the spray drying process, the surface composition of powder is affected by the type and concentration of emulsifier or surfactant used in the system. During atomization for spraying droplets in a very short time, the droplets are expelled into chamber and contact with the hot air. The solvent is readily available at the droplet surface, giving rise to fast and constant rate evaporation with stable surface temperature. When a critical point is reached, the solvent availability at the surface is insufficient to maintain water saturated at the surface, and a crust is formed. During the process, the most surface-active components are absorbed in the droplet surface and thereby dominate the particle surface. The surfactant absorbs faster at and creates the new interface that decreases the surface free energy and tension of air/droplet resulting rapid evaporation of water and evaporative cooling at the surface. Therefore, bioactive compounds could be retained and powder was drying fast. Furthermore, surfactants also reduces interfacial tension of droplet with a chamber wall, hence minimizes the stickiness [23–25]. Therefore, process yield of sample containing emulsifier was higher than sample without Tween 80.

Table 2 Process yield (%) and physical properties of spray-dried encapsulated curcumin powders

Tween 80/ Chitosan ratio (%w/w)	Process yield (%)	Physical properties of powders		
		Moisture content (%)	Water activity	Water solubility index (%)
1.0/0.0	40.20±2.76[a]	1.87±1.34[a]	0.119±0.001[a]	84.10±4.98[ab]
1.0/1.0	41.17±2.17[a]	2.51±1.24[a]	0.121±0.084[a]	86.50±1.09[a]
1.0/2.0	37.56±3.51[ab]	2.53±0.61[a]	0.140±0.002[a]	86.15±2.19[a]
1.0/3.0	33.02±0.21[b]	2.44±0.09[a]	0.143±0.026[a]	79.64±3.66[ab]
0.0/1.0	25.91±3.52[c]	1.80±0.97[a]	0.167±0.004[a]	75.15±1.06[b]

Different letters on superscript within a column were indicated that the values with significant differences ($p < .05$)

The increase of the chitosan concentration decreased the process yield ($p<0.05$). The higher the level of chitosan in the curcumin emulsion, the higher viscosity. The higher viscosity of the feed emulsion resulting in larger particles that were likely to deposit and stick in the chamber, and hence it will reduce process yield [26]. When the larger droplets are produced, thus creating a lower total area for water evaporation. Therefore, the liquid feed requires more temperature for drying, this lead to semi-wet powder that would stick at dryer chamber. Powder stickiness is mainly affecting process yield, it is also correlated with application of drying temperature near the glass transition temperature (*Tg*). The recommendation of outlet temperature used should be below *Tg* for minimize stickiness [27].

The water solubility index of the encapsulated curcumin powder was affected by the presence of a surfactant ($p<0.05$). The presence of Tween 80 improved the solubility

* Corresponding author e-mail: chaleedab@hotmail.com, chaleeda.b@chula.ac.th
[1]ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
[2]หน่วยปฏิบัติการวิจัยกระบวนการผลิตเพื่อออกแบบสมบัติเชิงหน้าที่ของอาหาร ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
[3]ภาควิชาวิศวกรรมเคมี คณะวิศวกรรมศาสตร์และวิทยาศาสตร์ มหาวิทยาลัยมูฮัมหมัดดียา พูโวกาโต อินโดนีเซีย

of encapsulated curcumin because it reduced the surface tension during the dispersing liquid. The dispersion kinetics of the powder into the water was affected by the physical properties of the powder itself. The amount of emulsifier would be reduced surface tension between powder and liquid that was facilitating powders immerse into water [28]. All samples with emulsifier and chitosan showed good solubility (> 80%). The addition of Tween 80 and chitosan did not affect moisture content and water activity. Based on water activity results showed that all encapsulated curcumin powder samples indicated microbial safe food products due to a_w less than 0.6 [29].

The micrograph of encapsulated curcumin powders was presented in SEM images (Figure 1). All encapsulated samples revealed non-homogeneous particle sizes (A1, B1, C1). This variability on the particle size was due to the instability of feed emulsion. Some coalescence droplets resulted in the larger size of microencapsulated powders. The particle of a sample with the addition of only Tween 80 seemed to the more spherical shape and less wrinkled surface (A2 compared to B2). Without the addition of Tween 80, the encapsulated powder showed agglomeration, clumping, and irregular shape (C2). However, the addition of chitosan resulted in a less spherical shape and smooth surface (B2). The possible explanation was that chitosan helps the protection of oil droplets from heat during drying. The protection was caused by the cationic of an amine group of chitosan that interacts with the polar head of oil. This protection increase curcumin that encapsulates into powder, thus increase the encapsulation efficiency. Chitosan protects the droplet from heat during fast evaporation resulted in the wrinkled on the surface of the powder and more irregular shapes [6].

The *L** (lightness), *a** (green-red) and *b** (blue-yellow) color value of spray-dried encapsulated curcumin powder are presented in Table 3**.** The *L**, *a**, and *b** values of all the samples were comparable except for powder without the addition of chitosan. Without chitosan, the sample showed more yellowness and brighter. The addition of chitosan decreased the yellowness of the encapsulated curcumin powder and also a darker color that contributed from the brown chitosan powder [6]. The increasing concentration of chitosan did not affect the color after that.

* Corresponding author e-mail: chaleedab@hotmail.com, chaleeda.b@chula.ac.th
[1]ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
[2]หน่วยปฏิบัติการวิจัยกระบวนการผลิตเพื่อออกแบบสมบัติเชิงหน้าที่ของอาหาร ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
[3]ภาควิชาวิศวกรรมเคมี คณะวิศวกรรมศาสตร์และวิทยาศาสตร์ มหาวิทยาลัยมูฮัมหมัดดียา พูโวกาโต อินโดนีเซีย

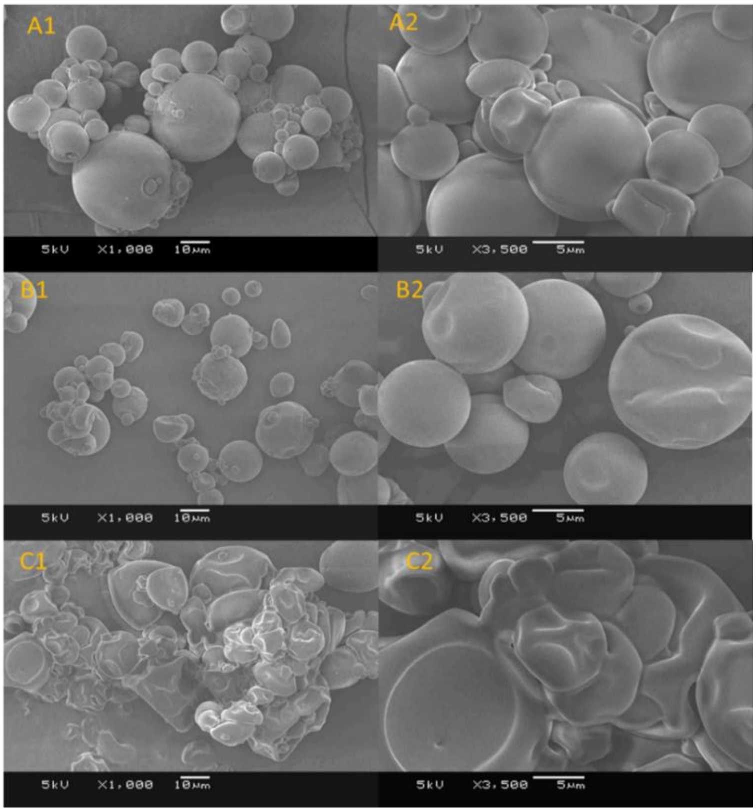

Figure 1 SEM images of encapsulated curcumin powders; Tween 80/Chitosan ratio 1/0 at 1000 × (A1), 3500 × (A2); ratio 1/1 at 1000 × (B1), 3500 × (B2); ratio 0/1 at 1000 × (C1), 3500 × magnification (C2)

Table 3 The CIELAB color space (*L**, *a** and *b**) of spray-dried encapsulated curcumin powder

Tween 80/chitosan ratio (w/w)	*L**	*a**	*b**
1/0	77.69 ± 0.11^{a}	-13.77 ± 1.10^{b}	55.08 ± 1.94^{a}
1/1	70.09 ± 3.22^{b}	-7.40 ± 1.28^{a}	47.81 ± 1.31^{b}
1/2	69.68 ± 0.28^{b}	-5.87 ± 0.51^{a}	45.67 ± 3.47^{b}
1/3	69.68 ± 0.32^{b}	-5.87 ± 0.40^{a}	45.67 ± 0.39^{b}
0/1	70.70 ± 1.57^{b}	-6.22 ± 0.86^{a}	45.63 ± 0.89^{b}

Different letters on the superscript within a column were indicated that the values with significant differences ($p < .05$)

3. Encapsulation efficiency and curcumin concentration

The addition of both Tween 80 and chitosan into the samples was affecting the TCC of encapsulated curcumin powders (Table 4). During the preparation of feed emulsion, the non-polar curcumin was strongly interacted with chitosan. The interaction was stronger in the existing surfactants. Boruah et al. (2012) reported that the interaction in that binding are hydrophobic, electrostatic, and hydrogen bond [30]. During the spray drying process, enhanced binding interaction of Tween80 and chitosan might protect oil containing curcumin from heat, resulting in better curcumin retention in the final product [26].

* Corresponding author e-mail: chaleedab@hotmail.com, chaleeda.b@chula.ac.th
[1]Department of Food Technology, Faculty of Science, Chulalongkorn University
[2]Emerging Process for Food Functionality design (EPFFD) research unit, Department of Food Technology, Faculty of Science, Chulalongkorn University
[3]Department of Chemical Engineering, Faculty of Engineering and Science, Universitas Muhammadiyah Purwokerto, Indonesia

Table 4 Curcumin concentration and encapsulation efficiency (EE) of encapsulated curcumin powder at different Tween80/Chitosan ratio.

Tween80/Chitosan ratio (%w/w)	Curcumin concentration (mg/g)		Encapsulation efficiency (%)
	Total curcumin content (TCC)	Surface curcumin content (SCC)	
1/0	0.79± 0.11^{bc}	0.48± 0.02^{b}	39.16±3.31^{b}
1/1	0.99± 0.09^{a}	0.45± 0.06^{b}	57.43±4.56^{a}
1/2	0.85± 0.12a^{bc}	0.38± 0.01^{b}	52.42±0.58^{ab}
1/3	0.69± 0.07^{c}	0.37± 0.15^{b}	40.88±5.54^{b}
0/1	0.90± 0.06^{ab}	0.73± 0.01^{a}	18.23±1.44^{c}

Different letters were indicated that the values with significant differences (p < 0.05)

The presence of a surfactant affected SCC. The results showed that SCC for encapsulated powders sample without emulsifier was significantly higher than other groups (p<0.05). Tween 80 as a non-ionic surfactant in the system reduced the interfacial tension of oil/water in the emulsion, and it would facilitate the interfacial liquid/air during drying. Without an emulsifier, curcumin was not effectively encapsulated, and hence the oils stayed on the surface of powders [25, 31]. Generally, the addition of emulsifiers also reduced oil droplet size of feed emulsion [32]. It led to uniform distribution of small oil droplets embedded in the emulsion droplet. During atomizer, the bigger droplet tends to break up and eventually the contact time of drying was decreased. Therefore, curcumin could be retained in the microcapsule. Hence, using Tween 80 combined with chitosan and maltodextrin was more powerful to encapsulate curcumin than using only chitosan and maltodextrin.

Our result showed that the addition of the combination of Tween 80 and chitosan improved encapsulation efficiency (EE) (Table 4). The use of non-ionic surfactant and chitosan increased curcumin protection during drying. The sample without added Tween 80 demonstrated the lowest EE because it had a higher SCC. Emulsifier not only facilitated the reducing of interfacial tension between oil in water in feed emulsion but also helped to minimize interfacial air and liquid as explained previously. Therefore, the existence of a surfactant protects the bioactive compound from the rapid evaporator as the surface free energy was decreased. The presence of chitosan might protect oil droplets that eventually enhanced curcumin retention [33]. However, the increase of chitosan concentration, the EE value was getting down. The higher concentration of chitosan use, the higher viscosity of feed emulsion resulted in bigger drops during atomizer, hence longer contact time of drying, therefore more degradation of compounds [24, 34]. There was another report that used the chitosan and Tween 20 as carrier agents in the encapsulation of curcumin spray-

* Corresponding author e-mail: chaleedab@hotmail.com, chaleeda.b@chula.ac.th
[1]Department of Food Technology, Faculty of Science, Chulalongkorn University
[2]Emerging Process for Food Functionality design (EPFFD) research unit, Department of Food Technology, Faculty of Science, Chulalongkorn University
[3]Department of Chemical Engineering, Faculty of Engineering and Science, Universitas Muhammadiyah Purwokerto, Indonesia

dried powder. The results also exhibited that the addition of both ingredients improved encapsulation efficiency [6].

4. Evaluation of antioxidant activity

The antioxidant activities determined used DPPH, and FRAP assays were in mM Trolox equivalent/g dry based (Figure 2). Our result indicated there was not significant difference in antioxidant activity in the DPPH assay (p<0.05). the addition of the combination of Tween 80 and chitosan slightly increased the antioxidant activity of encapsulated powders in the DPPH assay. These results were confirmed by the TCC results in the previous part. The chitosan at low concentration (ratio 1/1) produced better protection to curcumin during the drying process compared to that at a higher level. The higher concentration of chitosan increased the viscosity that eventually affected the process yield and reduce antioxidant activity as explained previously.

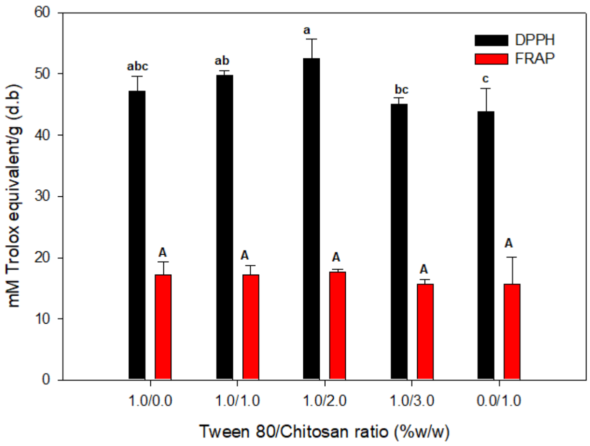

Figure 2 Antioxidant activity (DPPH and FRAP methods in mM Trolox equivalent (TE)/g) of encapsulated curcumin powder at different Tween 80/ Chitosan ratio.
Different letters were indicated that the values with significant differences (p < 0.05)

The results also showed that the results of antioxidant activity by DPPH had higher than the FRAP assay. It might be explained that the dominant antioxidant activity mechanisms of curcumin as hydrogen atom transfer (HET). Several reports had proposed about these mechanisms that the inhibition of lipid peroxidation and free radical scavenging capability of curcumin were an electron donation of the phenolic_part [35, 36]. Also, another report had attributed that the antioxidant activity mechanism relates H-atom donation in the heptadienone part in the central active CH_2 group of curcumin keto form [37]. However, it was explained that in the over-all reactions as an antioxidant, the abstraction of a hydrogen atom from the phenolic group in the side has a significant contribution [36].

CONCLUSION

This study determined the effect of combination of surfactant and emulsifier on encapsulation of curcumin via spray drying process by varying the ratio concentration of

* Corresponding author e-mail: chaleedab@hotmail.com, chaleeda.b@chula.ac.th
[1]ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
[2]หน่วยปฏิบัติการวิจัยกระบวนการผลิตเพื่อออกแบบสมบัติเชิงหน้าที่ของอาหาร ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
[3]ภาควิชาวิศวกรรมเคมี คณะวิศวกรรมศาสตร์และวิทยาศาสตร์ มหาวิทยาลัยมูฮัมหมัดดียา พูโวกาโต อินโดนีเซีย

the combination of Tween 80 – chitosan at a fixed amount of 20 %w/w of maltodextrin as wall material. The addition of Tween 80 and chitosan affected the physical and morphological properties of resulted curcumin powders as they could increase encapsulation efficiency and bioactivity. The lower concentration of chitosan combined with Tween 80 (ratio 1/1) exhibited the highest %EE. However, the EE values were <60% due to the emulsions were not stable and protected, and the use of mini spray dryer. At the best condition, chitosan combined with Tween 80 (ratio 1/1), the encapsulated curcumin powders also possessed the highest antioxidant activity. Thus, it can be concluded that the addition of mixed combination Tween 80 – chitosan as an encapsulation agent at ratio 1/1(%w/w) had the highest EE and antioxidant activity. Further study needs to investigate the approach to increase %EE by focusing more on maintaining the stability of feed emulsion, spray drying conditions, and wall materials.

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REFERENCES

[1] Xu, X. Y., Meng, X., Li, S., Gan, R., Li, Y., and Li, H-B. (2018). Bioactivity, health benefits, and related molecular mechanisms of curcumin: Current progress, challenges, and perspectives. Nutrients. 10(10): 1553 -1596. doi:10.3390/nu10101553

[2] Ahmed, K., Li, Y., McClements, D. J., and Xiao, H. (2012). Nanoemulsion- and emulsion-based delivery systems for curcumin: Encapsulation and release properties. Food Chemistry. 132(2): 799–807. doi:10.1016/j.foodchem.2011.11.039

[3] Ballesteros, L. F., Ramirez, M.J., Orrego, C.E, Teixeira, J.A., and Mussatto, S. I. (2017). Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials. Food Chemistry. 237: 623–631. doi:10.1016/ j.foodchem.2017.05.142

[4] Shishir, M. R. I., and Chen, W. (2017). Trends of spray drying: A critical review on drying of fruit and vegetable juices. Trends in Food Science & Technology. 65: 49–67. doi: 10.1016/j.tifs.2017.05.00

[5] Li, J., Hwang, I.C., Chen, X., and Park, H. J. (2016). Effects of chitosan coating on curcumin loaded nano-emulsion: Study on stability and in vitro digestibility. Food Hydrocolloids. 60: 138–147. doi:10.1016/ j.foodhyd.2016.03.016

[6] O'Toole, M.G., Henderson, R. M., Soucy, P. A., Fasciotto, B. H., Hoblitzell, P. J., Keynton, R. S., Ehringer, W. D., and Gobin, A. S. (2012). Curcumin encapsulation in submicrometer spray-dried chitosan/tween 20 particles. Biomacromolecules. 13: 2309–2314. doi:10.1021/bm300564v

[7] Deng, L., Kang, X., Liu, Y., Feng, F., and Zhang, H. (2017). Effects of surfactants on

* Corresponding author e-mail: chaleedab@hotmail.com, chaleeda.b@chula.ac.th
[1]ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
[2]หน่วยปฏิบัติการวิจัยกระบวนการผลิตเพื่อออกแบบสมบัติเชิงหน้าที่ของอาหาร ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
[3]ภาควิชาวิศวกรรมเคมี คณะวิศวกรรมศาสตร์และวิทยาศาสตร์ มหาวิทยาลัยมูฮัมหมัดดียา พูโวกาโต อินโดนีเซีย

the formation of gelatin nanofibres for controlled release of curcumin. Food Chemistry. 231: 70–77. doi:10.1016/ j.foodchem.2017.03.027

[8] McCements, D. J., and Decker, E. (2018). Interfacial antioxidants: A review of natural and synthetic emulsifiers and coemulsifiers that can inhibit lipid oxidation. Journal of Agricultural and Food Chemistry. 66: 20–35. doi:10.1021/acs.jafc.7b05066

[9] Kharat, M., Du, Z., Zhang, G., and McClements, D. J. (2017). Physical and chemical stability of curcumin in aqueous solutions and emulsions: Impact of pH, temperature, and molecular environment. Journal of Agricultural and Food Chemistry. 65: 1525–1532. doi:10.1021/acs.jafc. 6b04815

[10] Dantas, D., Pasquali, M. A., Cavalcanti-Mata, M., Duarte, M. E., and Lisboa, H. M. (2018). Influence of spray drying conditions on the properties of avocado powder drink. Food Chemistry. 266: 284–291. doi:10.1016/ j.foodchem.2018.06.016

[11] AOAC. (2016) Official methods of analysis of AOAC International (20th ed). AOAC International, Rockville, Maryland, USA.

[12] Martins, R. M., Pereira, S. V., Siqueira, S., Salomão, W. F., and Freitas, L. A. P. (2013). Curcuminoid content and antioxidant activity in spray dried microparticles containing turmeric extract. Food Research International. 50: 657–663. doi:10.1016/ j.foodres.2011.06.030

[13] Ahmed, M., Akter, M. S., Lee, J. C., and Eun, J. B. (2010). Encapsulation by spray drying of bioactive components, physicochemical and morphological properties from purple sweet potato. LWT-Food Science & Technology. 43: 1307–1312. doi:10.1016/ j.lwt.2010.05.014

[14] Laokuldilok, N., Thakeow, P., Kopermsub, P., and Utama-Ang, N. (2015). Optimisation of microencapsulation of turmeric extract for masking flavour. Food Chemisty. 194: 695–704. doi:10.1016/j.foodchem.2015.07. 150

[15] Brand-Williams, W., Cuvelier, M. E., and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. Lebensmittel Wissenschaft und Technologie. 28: 25–30.

[16] Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., and Byrne, D. H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis. 19: 669–675. doi:10.1016/j.jfca.2006.01.003

[17] Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. Progress in Polymer Science. 31: 603–632. doi:10.1016/ j.progpolymsci.2006.06.001

[18] El-Hefian, E. A., Khan. R. A., and Yahaya A. H. (2008). Study of the parameters affecting the viscosity of chitosan solution. Journal Chemical Society of Pakistan.30(4): 529–531

[19] Li, Y., Ai, L., Yokoyama, W., Shoemaker, C. F., Wei, D., Ma, J., and Zhong, F. (2013). Properties of chitosan-microencapsulated orange oil prepared by spray-drying and its stability to detergents. Journal of Agricultural and Food Chemistry. 61: 3311–3319. doi:10.1021/jf305074q

* Corresponding author e-mail: chaleedab@hotmail.com, chaleeda.b@chula.ac.th

[1]ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

[2]หน่วยปฏิบัติการวิจัยกระบวนการผลิตเพื่อออกแบบสมบัติเชิงหน้าที่ของอาหาร ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

[3]ภาควิชาวิศวกรรมเคมี คณะวิศวกรรมศาสตร์และวิทยาศาสตร์ มหาวิทยาลัยมูฮัมหมัดดียา พูโวกาโต อินโดนีเซีย

[20] Klaypradit, W., and Huang, Y. W. (2008). Fish oil encapsulation with chitosan using ultrasonic atomizer. LWT - Food Science and Technology. 41:1133–1139. doi:10.1016/j.lwt.2007.06.014

[21] Chuacharoen, T., Prasongsuk, S., and Sabliov, C. M. (2019). Effect of surfactant concentrations on physicochemical properties and functionality of curcumin nanoemulsions under conditions relevant to commercial utilization. Molecules. 24: 2744-2756. doi:10.3390/molecules24152744

[22] Akolade, J. O., Oloyede, H. O. B., and Onyenekwe, P. C. (2017). Encapsulation in chitosan-based polyelectrolyte complexes enhances antidiabetic activity of curcumin. Journal of Functional Foods. 35: 584–594. doi:10.1016/j.jff.2017.06.023

[23] Nuzzo, M., Millqvist-Fureby, A., Sloth, J., and Bergenstahl, B. (2015). Surface composition and morphology of particles dried individually and by spray drying. Drying Technology. 33(6): 757–767. doi:10.1080/07373937.2014.990566

[24] Munoz-Ibanez, M., Nuzzo, M., Turchiuli, C., Bergenståhl, B., Dumoulin, E., and Millqvist-Fureby, A. (2016). The microstructure and component distribution in spray-dried emulsion particles. Food Structure. 8: 16–24. doi:10.1016/j.foostr.2016.05.001

[25] Drapala, K. P., Auty, M. A. E., Mulvihill, D. M., and O'Mahony, J. A. (2017). Influence of emulsifier type on the spray-drying properties of model infant formula emulsions. Food Hydrocolloids. 69: 56–66. doi:10.1016/j.foodhyd.2016.12.024

[26] Kumar, L. R. G., Chatterjee, N. S., Tejpal, C. S., Vishnu, K. V., Anas, K. K., Asha, K. K., Anandan, R., and Mathew, S. (2017). Evaluation of chitosan as a wall material for microencapsulation of squalene by spray drying: Characterization and oxidative stability studies. International Journal of Biological Macromolecules. 104: 1986–1995. doi:10.1016/j.ijbiomac.2017.03.114

[27] Normand, V., Subramaniam, A., Donnelly, J. and Bouquerand, P. E. (2013). Spray drying: Thermodynamics and operating conditions. Carbohydrate Polymers. 97: 489–495. doi:10.1016/j.carbpol.2013.04.096

[28] Liu, W., Chen, X. D., Cheng, Z., and Selomulya, C. (2016). On enhancing the solubility of curcumin by microencapsulation in whey protein isolate via spray drying. Journal of Food Engineering. 169: 189–195. doi:10.1016/j.jfoodeng.2015.08.034

[29] Petruzzi, L., Corbo, M. R., Sinigaglia, M., and Bevilacqua, A. (2017). Microbial spoilage of foods: Fundamentals. The microbiological quality of food ed by Bevilacqua, A., Corbo, M.R., and Sinigaglia, M. Elsevier Ltd. doi:10.1016/B978-0-08-100502-6.00002-9

[30] Boruah, B., Saikia, P. M., and Dutta, R. K. (2012). Binding and stabilization of curcumin by mixed chitosan-surfactant systems: A spectroscopic study. Journal of Photochemistry and Photobiology: A Chemistry. 245: 18–27. doi:10.1016/j.jphotochem.2012.07.004

[31] Munoz-Ibanez, M., Azagoh, C., Dubey, B. N., Dumoulin, E., and Turchiuli, C. (2015). Changes in oil-in-water emulsion size distribution during the atomization step in

* Corresponding author e-mail: chaleedab@hotmail.com, chaleeda.b@chula.ac.th
[1]ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
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[3]ภาควิชาวิศวกรรมเคมี คณะวิศวกรรมศาสตร์และวิทยาศาสตร์ มหาวิทยาลัยมูฮัมหมัดดียา พูโวกาโต อินโดนีเซีย

spray-drying encapsulation. Journal of Food Engineering. 167: 122–132. doi:10.1016/j.jfoodeng.2015.02.008

[32] Wu, M. H., Yan, H. H., Chen, Z. Q., and He, M. (2017). Effects of emulsifier type and environmental stress on the stability of curcumin emulsion. Journal of Dispersion Science and Technology. 38(10): 1375–1380. doi:10.1080/01932691.2016.1227713

[33] Neves, M. I. L., Desobry-Banon, S, Perrone, I. T., Desobry, S., and Petit, J. (2019). Encapsulation of curcumin in milk powders by spray-drying: Physicochemistry, rehydration properties, and stability during storage. Powder Technology. 345: 601–607. doi:10.1016/j.powtec.2019.01.049

[34] Schröder, J., Kleinhans, A., Serfert, Y., Drusch, S., Schuchmann, H. P., and Gaukel, V. (2012). Viscosity ratio: A key factor for control of oil drop size distribution in effervescent atomization of oil-in-water emulsions. Journal of Food Engineerig. 111: 265–271. doi:10.1016/j.jfoodeng.2012.02.023

[35] Barzegar A. (2012). The role of electron-transfer and H-atom donation on the superb antioxidant activity and free radical reaction of curcumin. Food Chemistry. 135: 1369–1376. doi:10.1016/j.foodchem.2012.05.070

[36] Priyadarsini, K. I., Maity, D. K., Naik, G. H., Kumar, M. S., Unnikrishnan, M. K., Satav, J. G., and Mohan, H. (2003). Role of phenolic O-H and methylene hydrogen on the free radical reactions and antioxidant activity of curcumin. Free Radical Biology & Medicine. 35(5): 475–484. doi:10.1016/S0891-5849(03)00325-3

[37] Jovanovic, S. V., Steenken, S., Boone, C. W., and Simic, M. G. (1999). H-atom transfer is a preferred antioxidant mechanism of curcumin. Journal of American Chemical Society. 121: 9677–9681. doi:10.1021/ja991446m

* Corresponding author e-mail: chaleedab@hotmail.com, chaleeda.b@chula.ac.th
[1]ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
[2]หน่วยปฏิบัติการวิจัยกระบวนการผลิตเพื่อออกแบบสมบัติเชิงหน้าที่ของอาหาร ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
[3]ภาควิชาวิศวกรรมเคมี คณะวิศวกรรมศาสตร์และวิทยาศาสตร์ มหาวิทยาลัยมูฮัมหมัดดียา พูโวกาโต อินโดนีเซีย