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Abstract. The application of catechin is limited due to low stability, however nanoencapsulation technology can improve catechin stability. This study aimed to determine the effect of the types and concentrations surfactants in the catechin encapsulation process on β-CD. Concentrations of surfactants used were Poloxamer 188 (NKP, 0.5%-1.5%), Tween 80 (NKT, 2,5% and 3%) without surfactants was used to control (NKB). The catechin nanoemulsion complex formed was inclusion them into β-CD (ratio 1:1), and dried by spray drying. The stability test showed a sample of NKP 1% was more stable with the lower turbidity and viscosity values, namely 175 NTU and 0.93 cP, NKT 2.5% (118 NTU and 0.94 cP), NKB (461 NTU and 4.0 cP). The size of the sample particle decreased according to an increase in the surfactant concentration, where the NKP 1% (37 nm) produced smaller particles, the appearance of clear and yellowish suspension. NKP 1% had the highest EE value, followed by that NKT 2.5%, and NKB, i.e. 91.9%, 89.5%, 77.4%, respectively. Sample NKP 1% had a morphology shape with compact structures and the highest crystallinity degree (92.4%). This research showed the use of surfactants could improve the stability of catechins compared to that without surfactants.

1. Introduction

Uncaria gambier Roxb. (gambier) is one of the Indonesian community plantation commodities that has high economic value and perspective for multi-use commercial development in the future. Catechin is one of the main components of the gambier plant. Its amount in gambier extract depends on the processing. Gambier contains catechin phenolic that has a role as an antioxidant [1,2].

Phenolic compounds have antioxidant properties by scavenging radical oxygen species [3–5]. The instability of catechin is shown by the change in its color from yellowish-white to brown when it is left

in the open air. The instability of its active compounds can decrease bioactivity during the storage so that the application process is still difficult to be applied [2,6,7].

Nanoencapsulation technology can improve bioavailability. It also can control and improve the release properties of the active compound [8–11]. Previous researchers have encapsulated active compounds of catechins using a variety of different methods and coatings. But no one has researched the catechin encapsulate from gambier catechin raw materials and uses a size reduction method through the process of emulsification using surfactants Poloxamir 188 and Tween 80 before being coated on β-cyclodextrin (β-CD).

The technology of the encapsulation matrix method of inclusion with β-CD has become a popular method in increasing the stability and solubility of poorly soluble active compounds.This method has a unique characteristic β-CD, as host, has a cavity, in which the outer surface is hydrophilic and the inner surface is lipophilic (hydrophobic). This characteristic makes it possible to select active compounds (guest) to be inserted [6,12–14].

The formation of the complex inclusion to produce stable products requires high efficiency of active compound adsorption into β-CD. Adsorption of active compounds into the β-CD can be done if the guest molecules are significantly smaller than the cavity of β-CD. An active compound with nano-size can get inclusion into β-CD properly because it possesses a large surface so that it can be absorbed into the β-CD perfectly and can improve the stability of the active compounds [6,7,15,16].

To produce nanoparticle of catechin from gambier, a processing technology that does not damage the particles is needed. The process of emulsification of nanoparticle catechin using surfactants can increase the stability of emulsion products. Surfactants have good control during nano-capsulate formation. The surfactants have a function to reduce surface tension and interfacial tension. The emulsion process to produce nano-sized particles is affected by current power stirring [15,17–20].

Increased catechin stability was needed in storage and application. Therefore, it is needed to find the appropriate type of surfactant to produce catechin nanoencapsulation products from gambier which was stable in storage. This study aimed to determine the effect of the types and concentrations of Poloxamer 188 and Tween 80 surfactants in the catechin encapsulation process on β-CD and analysed the potential nanoemulsion-based inclusion complexes formed that can increase its stability.

2. Experimental

2.1. Materials
Catechin from gambier was made used the method of [2] β-cyclodextrin, and Tween 80 (Sigma-Aldrich), Polaksamer 188 (PT. BASH Indonesia), distilled water, technical ethanol, deionized water were purchased from Betrachem-Bogor, chemicals for analysis by Merck of Darmstadt, Germany.

2.2. Methods
2.2.1. Determination of the encapsulation efficiency of nanoencapsulation of catechin- β-cyclodextrin.
Nanoencapsulated catechin (25 mg) was dissolved in 50 mL buffer phosphate pH 7.2. The mixture was shaken for 24 hours and filtered. The filtrate's absorbance was read using a UV-VIS spectrophotometer, at the maximum wavelength was (λ) 279 nm. Encapsulation efficiency (EE) was calculated by the equation 1.

$$EE = \frac{x \text{ mgL}^{-1} \text{ x 1 L x 1000 mL}^{-1} \text{x vol.extraction x a mg x b mg}^{-1}}{\text{mass of first catechin (mg)}} \text{ x 100\%} \tag{1}$$

x is concentration of formula, a is total mass of nanoparticles that obtained, and b is mass of nanoparticles used for the determination of efficiency.

2.2.2. Production of encapsulation of catechin in β-cyclodextrin without surfactants.
Nanoencapsulate of catechin without surfactants was made using a modified method by [21]. Catechin was dissolved in ethanol 70% (1:10), stirred with a magnetic stirrer at 50 rpm for 5 min and then sonicated for 5

minutes. β-cyclodextrin was diluted with distilled water whose temperature is 50±5°C (4:1), stirred with a magnetic stirrer at 700 rpm for 5 minutes. Catechin solution was inclusion on the β-CD (molar ratio of 1:1) with a magnetic stirrer for 24 hours. The complex inclusion solution of catechin-β-CD evaporated its solvent with spray dryer at the condition of inlet temperature of 175±5°C and the outlet temperature of 90±5°C, a solution flow rate 10 mL.min^{-1}.

2.2.3. Production of encapsulation of catechins in β-cyclodextrin through an emulsion process. The making of catechin nanoencapsulation with surfactants used a modified method by [15], [22]. β-cyclodextrin (40 mg) was dissolved in 10 mL of deionized water at a temperature 50±5°C (water phase). Nanoemulsion catechin used surfactants Poloxamer (NKP), Tween surfactant (NKT) and without surfactant (NKB) were dropped on β-CD (3 mL.min^{-1}) while stirred with a magnetic stirrer (700 rpm). After combining the nanoemulsion into β-CD, it is continued with magnetic stirring at 400 rpm for 18±2.0 hours. Inclusion complex of catechin-β-CD which was formed from the evaporated solvent with a spray dryer at an inlet temperature of 175±5°C, the outlet temperature of 80±5°C and flow rate solution 10 mL.min^{-1}.

3. Results and discussion

3.1. Preparation of nanoencapsulation catechin conventionally (without surfactants)

The results showed that particle preparation of catechin from gambier used in this process produced catechin, with an average diameter particle size was big enough (607 nm), with low homogeneity polydispersity index (IP) 2.96 (table 1). The polydispersity index indicated the size of the mass distribution of molecules in the sample. IP value was less than 0.3 indicating that the sample has a size distribution which is more uniform or homogeneous [20,24,25].

The process of micelles formation by emulsions surfactants and particle size that was produced was strongly influenced by the mixing process or ultrasonication. Formation of catechin nanoemulsion was conducted by using a surfactant Poloxamer 188 ($HO(C_2H_4O)_{38}(C_3H_6O)_{29}(C_2H_4O)_{38}H$) and Tween 80 ($C_{64}H_{124}O_{26}$). The results of emulsion treatment with the smallest particle size catechin will be included on β-CD.

The process of making nanoencapsulation with active compounds by inclusion is influenced by the composition of the materials and methods used. The composition of a material affects the equilibrium complex formation of active compounds. The composition of material between catechin and β-CD equilibrium formation of inclusion complex occur in comparison molarity of 1:1, wherein catechin used was 300 mg (molecular weight of catechin = 290.26 g. mol^{-1}), and β-CD was 1144 mg (molecular weight β-CD=1134.99 g.mol^{-1}). In this condition, the mole fraction of catechin bounded with β-CD was 0.85 and the value of fixed constant (Ka.M^{-1}) was 21.800 M^{-1} [13,15,21].

Table 1. The results of research formation nanoencapsulation of catechin conventionally (without surfactants).

Process	Turbidity (NTU	Viscosity (cP)	Mean of particle size diameter (nm)	Diameter (nm)			IP
				(10%)	(50%)	(90%)	
Preparation of catechin solution							
Stirring 700 rpm, 30 minutes, sonication 10 minutes	461	7.36	607	1 699	4 679	8 915	2.96
Formation of the catechin-β-CD inclusion complex							
Stirring 50 rpm for 24 hours	461	5.17	286	339	977	2 985	0.92

The reduction of particle size of catechin (tabel 1) can be done using ultrasonication technique and surfactants. Ultrasonic phase will reduce the particle size and the big energy of ultrasonic will be able to break down particles into smaller sizes and uniform. The weakness of using the ultrasonic technique is that long processing times, can produce heat and cause a decreasing activity of catechin. At the phase of the ultrasonication process, is where surfactants had a function to make effective collisions between molecules so that the large particles broke down into smaller (nano) [13,22].

Surfactants in reducing particle size depended on the type and concentration of it that used. Surfactants have surface activity (surface-active agent) which can reduce the surface tension between the air-liquid and liquid-liquid. The role of surfactants in lowering the interfacial tension with the surrounding particles took place during the merging process of surfactant (liquid phase) and the active compound (organic phase) through dropping. To produce nanoemulsion surfactants were dispersed by adjusting the number of droplets of the organic phase to the liquid phase [17,22,26,27].

The particle size affected the absorption efficiency of the quest into β-CD. To produce the perfect complexation with β-CD the size of the active compound molecules had to be smaller than the cavity of β-CD. The cavity of β-CD had a diameter of about 0.6-0.65 nm and volume about ±26.2 nm. Guest molecules which were larger than the β-CD cavity would result in the low and weak form of the complexes [7,13]. Large particle size also caused catechin molecules encapsulated in β-CD to become low (tabel 1). To improve the encapsulation high efficiency on the formation of catechin nanoencapsulation-β-CD a decreasing particle size of catechin was conducted.

3.2. Preparation of nanoencapsulation catechin conventionally (without surfactants).

3.2.1. Production of catechin nanoemulsion with surfactant. Production of catechin nanoemulsion done by phase inversion method was with the addition of the organic phase (catechin in 70% ethanol solution) into the liquid phase (surfactant and deionized water). The results of the catechin nanoemulsion analysis are shown in table 2.

The test results of emulsions (table 2) showed that the smallest particle size was obtained from the use of Poloxamer 1% (NKP) and Tween 2.5% (NKT) concentration, with an average diameter of the particle, sizes 84 nm (IP = 0.29) and 22 nm (IP = 0.25). A decreasing particle size predicted the value of turbidity, viscosity and particle size diameter (nm) which were produced. The particle size was directly proportional to the turbidity value, the smaller the particle size the lower the turbidity value of the suspension and the lower the viscosity the smaller the particle size.

Table 2. Value of turbidity, viscosity, particle size diameter (nm) and polydispersity index (IP) emulsion catechin.

Type of surfactants	Turbidity (NTU)	Viscosity (cP)	Mean of particle size diameter (nm)	Diameter (nm)			IP
				(10%)	(50%)	(90%)	
Poloxamer 188 (%)							
0.5	461	4.0	185	135	204	295	0.47
1	175	0.93	84	17	36	93	0.29
1.5	185	2.67	166	98	170	295	0.11
Tween 80 (%)							
1	461	6.4	Solution was turbid and had sediment				
1.5	461	5.7	Solution was turbid and had sediment				
2	406	3.0	245	246	676	1 863	0.75
2.5	118	0.94	22	7	11	21	0.25
3	199	1.17	151	41	170	490	0.37

Reduction size of the catechin particles at the ultrasonication stage due to the effects of cavitation produced vibration energy of sound waves spreading through the liquid medium in a cycle of high and low pressure (frequency of 20-40 kHz), simultaneous collision among the particles. At low pressure, bubbles were formed. They absorbed energy and then broke out at high pressure and released high of mechanical and thermal energy. The outbreak of the bubble caused the particle size became smaller and decreased the value of turbidity size [5,20,25,28,29].

The use of Poloxamer or Tween at lower concentration produced larger particle size with a high value of turbidity and viscosity (tabel 2). This condition might be due to the equilibrium between the surfactant and catechin that has not happened yet. High concentration surfactant produced a high value of turbidity and viscosity but the emulsion that was formed occurred separately between 2 phases in storage. It was possibly caused by a complex form already exceed the concentration of critical micelle concentration (CMC).

Emulsions that had a large particle size occurred when the surfactant concentration was lower than CMC and the surfactant used was not enough sufficiently contribute to reducing the interfacial energy between the active compounds and solvents. Conversely, the use of high surfactant concentrations caused clustered particles to form aggregates and larger sizes. Increasing particle size indicated that the emulsion was not balanced due to a tendency to agglomerate [10,15,26].

3.2.2. The formation of inclusion nanoemulsion complex of catechin-β-cyclodextrin (β-CD). The result of the inclusion complex formation of catechin-β-CD using surfactants Poloxamer (NKP) (table 3) produced the clear suspension, yellowish color with the smallest average of particle size (37 nm). Meanwhile, the surfactant Tween (NKT) produced a color slightly darker and an average diameter of the catechin particles which was bigger (177 nm) than that of the first nanoemulsion (Tween concentration 2.5%) (22 nm). This showed that catechin with a complex Tween surfactant had an unstable emulsion. The nanoparticle has limitations, its size and large surface area can lead to aggregation of the particles and physical handling is difficult in liquid form [10,15].

The result of the formation of inclusion complexes of catechin-β-CD samples NKB (without surfactant) produced complex turbid solution and sedimentation. This could be due to the large size of catechin particles (254 m) and their less homogeneous so it caused catechin inclusion could not take place perfectly in β-CD. The formation of an inclusion complex of the active compound with β-CD could form sediment due to a lack of sufficient power of combining between the head groups of active compounds with NKB.

Table 3. Particle size distribution of inclusion nanoencapsulate complex catechin-β-CD based on treatment variations.

Sample	Mean of diameter (nm)	Diameter (nm)			IP
		(10%)	(50%)	(90%)	
NKB	254	123	324	676	0.39
NKP	37	7	12	28	0.28
NKT	177	65	214	408	0.27

Complex inclusion of active compounds with β-CD occurred because the availability of cavity ring-shaped donat with the outside part was hydrophilic and with the inside part was hydrophobic. A hydrophobic cavity was an ideal place for a molecule that was less soluble in water by protecting the hydrophobic part. Catechin was a compound that was less soluble in cold water. β-CD had a role in shaping complex host-guests, where most of the surfactant put the hydrophobic edge truss into the cavity of β-CD. At the time of formation of inclusion, a complex active compound with β-CD occurred because of the driving force by H-bonds and ionic repulsion between the head and tail. The magnitude of each impulse greatly affected the stability of the surfactant-β-CD solution that occurs [2,7,15,26].

3.3. Test of Formation Catechin Inclusion Complex with β-Cyclodextrin (β-CD)

3.3.1. Morphology and structure of nanoencapsulation particle of catechin. The results of the nanoencapsulation morphology test using SEM showed that catechin had an irregular form of crystal particles with large sizes (figure 1). NKB samples had irregular morphology with a wrinkled surface and empty capsules. This might happen during the release of active compounds while the solvent evaporated.

NKP samples showed a shape of surface which is spherical, dense and homogeneous. Meanwhile, the NKT samples had a rounded, solid, and a bit rough surface. It also showed a round structure that had a cavity or which is empty. The NKT cavity structure was allegedly occurred due to the influence of heat during the process of solvent evaporation.
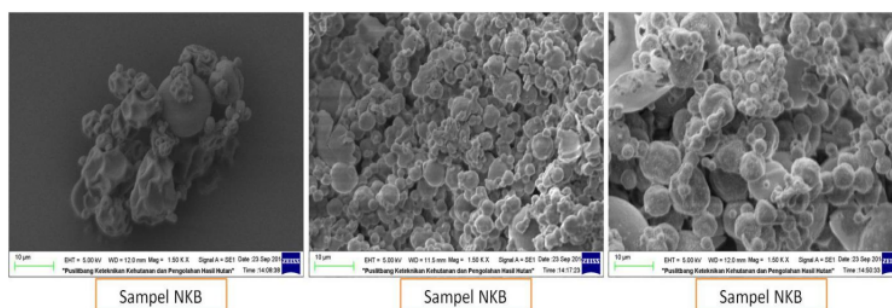

Figure 1. Morphology of nanoencapsulation catechin NKB, NKP and NKT result SEM photograph (1500X).

Complex morphology on the sample NKB could be attributed to the crimped rate of solvent evaporation during the drying process to generate heat spray dryer. This caused active compounds that were not resistant to heat disappeared or separated from the capsules. The samples with crimped morphology were possibly caused by the rapid evaporation of the points of fluid during the drying process using a spray dryer. The releasing of active compounds could be caused by large size particles which are trapped outside of the β-CD. The heat in the evaporation from the solvent could cause the re-releasing of active compounds of β-CD because the heat in the evaporation from solvent could occur re-releasing of the active compounds of β-CD [7,13].

3.3.2. Characteristics of stability of nanoencapsulate catechin. Evaluation of complex formation using XRD showed the diffraction pattern of the material of catechin powder (K sample) showed an amorphous structure with a low intensity of less crystal compared to that of NKB, NKP, and NKT samples. This characteristic was organic compounds and amorphous that showed the structure of unstable compounds. The lower the appeared top the intensity indicated the lower of its stability.

The diffraction pattern of NKB, NKP and NKT samples showed the process of complex formation which is seen from the form top (figure 2). The diffraction pattern of the NKB sample had the highest top intensity compared to that of NKT and NKB. However, the diffraction pattern of NKT and NKB was not significantly different in the crystal size. NKT sample gave a new climax at 2Θ 37.8, and NKP sample at 2Θ 37.9°. The new climax maybe came from each surfactant that was used.
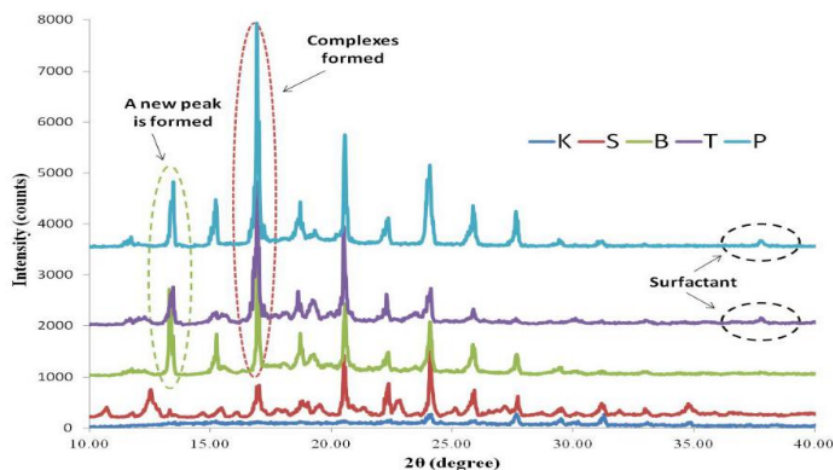
Figure 2. XRD patterns of catechin (K), β-cyclodextrin (S), NKB, NKP, and NKT.

Complex formation using XRD was done by observing the changing of the top particle of the guest molecule (active compounds). The appeared top in a complex can identify the characteristics of the sample and transition phase from a material as amorphous or crystal. The indication of complexation was a top with a narrow and sharp top. The higher the top the more complex the crystal. Low crystal was showed by imperfect complex and amorphous.

A new peak was found at XRD pattern, where the new climax maybe came from each surfactant that was used. It means there was an overlapping among the diffraction patterns of physical compounds, from active compounds and β-CD, with diffraction patterns that had lower climax intensity [11,19,27].

The results showed that the complex inclusion formation of catechin β-CD with surfactant could produce particle structure became more crystal. Nanoencapsulate catechin which was treated with NKP (table 4) produced crystal with a crystallinity degree in the highest degree and it indicated high stability as well.

Table 4. Crystallinity degree of raw materials and nanoencapsulation catechin.

Treatment	Number of total area (2Θ, 0° to 60°)	The area total (2Θ, 0° to 60°)	The degree of crystallinity (%)
Catechin (K)	41259	27342	66.3
NKB	81852	63161	77.2
NKP	93720	86957	92.4
NKT	79555	66609	83.7

3.3.3. Eficiency of catechin nanoencapsulate encapsulation. The results of EE analysis of catechin nanoencapsulate based on treatment variation showed that EE value from NKP sample was higher than that of the NKT and NKB, with treatment average values 91.9%, 89.5%, and 77.4% (figure 3). NKB sample had the lowest EE value, which may be due to the large particle size of catechin so that absorption of catechin at β-CD became lower too.
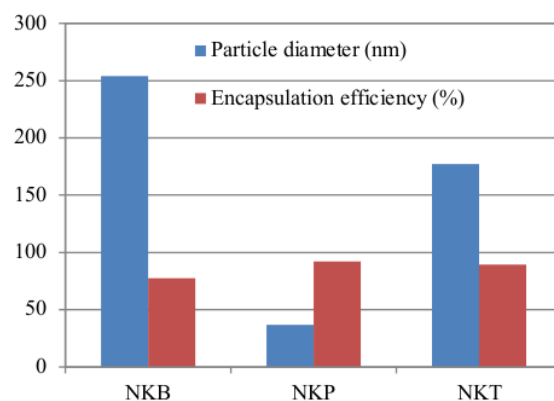
Figure 3. Effect of particle size to encapsulation efficiency
of catechin at β-cyclodextrin.

The particle size at catechin nanoencapsulate formation had opposite relation with EE. Samples with the highest EE value had a smaller particle size and can be more encapsulated at β-CD (NKP). In contrast, NKB samples with large diameter made a total catechin particle that could be included at β-CD became lower. The small number of catechin particle that got inclusion was showed by a separation between two phases in complex solution after the homogenization process. Sedimentation phase was predicted to contain incomplex catechin.

Analysis of EE of catechin nanoencpsulation was conducted to see the total amount of absobable catechin or those that were found inside the capsule. EE value of catechin nanoencapsulate directly affected the trapped catechin in β-CD got protection from environment influence (temperature, light). Particle size affected the EE of active compounds at β-CD. The smaller the particle size, the higher the EE value produced. This is because the small size of catechin particles can be included perfectly to β-CD. Particle size affected complex formation with β-CD. If guest molecules that were included were single molecules then the formed complex can change the physical characteristics and chemical molecules with higher solubility and stability.

The EE value can be influenced by the particle size of the active compound on β-CD. The smaller the particle size, the higher the EE value produced. This is because the small size of catechin particles can be included perfectly to β-CD. Particle size affected complex formation with β-CD. If guest molecules that were included were single molecules then the formed complex can change the physical characteristics and chemical molecules with higher solubility and stability [7,9,13].

Value of EE can be affected by the heat produced during the drying process and can result in a broken capsule. It was predicted that catechin were released from the capsule as it was seen from the morphology of NKT sample after SEM test. A temperature produced by the drying process can cause the loss or release of active compounds. This can be caused by the trapped particles outside of β-CD. The effect of the drying process is the release of active compounds which cause EE values lower [30,31].

4. Conclusion
Polaksamer 188 and Tween 80 surfactants functioned to form emulsions and reduce the particle size of catechin. Polaksamer 188 surfactants at a concentration of 1% produced the smallest and uniform particle size. Complex catechin nanoencapsulate inclusion of nanoemulsion with Poloxamer (NKP) at β-CD produced a stable complex solution with a particle size of 37 nm (IP 0.28). Catechin had the

highest nanoencapsulate efficiency value (91.9%) comparing using Tween (NKT) (89.5%) and without using surfactant (NKB) (77.4%). Complex inclusion formation using Poloxamer surfactant also produced nanoencapsulate, with the highest crystallinity degree (92.4%), and indicated the highest stability.

Acknowledgments

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