International Journal of Integrated Engineering – Special Issue on ReSENT 2017, Vol. 9 No. 4 (2017) p. 129-132

β-Cyclodextrin/Lecithin: Preparation and Characterization

Eng Hui Peng[1], Hatijah Basri[2,3*], Balkis A. Talip[1]

[1]Department of Technology and Natural Resources, [2]Department of Physics and Chemistry, Faculty of Applied Sciences and Technology, UTHM Pagoh Campus, 84600 Muar, [3]Microelectronics and Nanotechnology Shamsuddin Research Center (MiNT-SRC),
UTHM, 86400, Johor, MALAYSIA.

Abstract: Lecithin is a fat which is well-known to be obtained from soybeans or egg yolks. It is also taken as a medicine and used as food additive that keep certain ingredients from separating out. Despite of its advantages, lecithin's application in the industries is restricted due to its sensitivity to heat and light and can be easily oxidized. Hence, this study aims to investigate the effect of β-cyclodextrin (β-CD) in improving the properties of lecithin. The objectives of this study are to prepare and characterize β-CD/lecithin complex followed by study on the influence of β-CD on lecithin's thermal stability and antioxidant property. Analytical methods employed are fourier transform infra-red (FTIR) spectroscopy for chemistry study, differential scanning calorimetry (DSC) for examination of its thermal stability, DPPH testing for its antioxidant property as well as scanning electron microscopy (SEM) for structural examination. Results from FTIR and SEM analyses confirmed the formation of β-CD/lecithin complex. Thermal stability and antioxidant property of the complexes on the other hand were found to be improved. Of the amount of β-CD studied, it was reported that the best combination of the complex was at β-CD/lecithin ratio of 2:1. The complex of the optimized ratio showed the greatest thermal stability as indicated by its highest melting point (177.8 ºC). The complex also exhibited the greatest antioxidant property as indicated by its highest percentage of DPPH scavenging activity that is 97.1%.

Keywords: β-Cyclodextrin, Lecithin, Thermal stability

1.0 Introduction

Cyclodextrin (CD) is a low cost enzyme-modified starch derivative and cyclic oligosaccharide which can be used as a food ingredient owing to its non-toxicity [1]. It can be produced industrially through the enzymatic degradation of starch and has a wide range of applications in the food industry. Since 1998, β-cyclodextrin (β-CD) has obtained the generally rated as safe (GRAS) status which allows it to be added into the food without any concern.

Lecithin is widely used in the food, pharmaceutical and cosmetic industries as it can provide many health benefits to the consumers such as delaying of aging process, regulation of blood lipid, lowering of cholesterol, enhancement of memory as well as the prevention and treatment of diabetes [2]. It acts as a very important physiological activator and natural surface active agent. Lecithin can be obtained from soybeans owing to its availability and the outstanding functionalities [3].

However, its application in the industries is restricted as it is sensitive to heat and light and can be easily oxidized. Overall, even though lecithin and CD have significant contribution in food industries, the report of each or combined ingredients are very limited.

1.1 Materials and complex preparation

Commercially available soybean lecithin was obtained and utilized in this experiment while β-CD (≥ 97%) was purchased from Sigma-Aldrich. Ethanol and distilled water were used in the preparation of β-CD/lecithin complex whereas 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ascorbic acid were used in the DPPH radical scavenging assay.

To synthesize β-CD/lecithin complex, β-CD was first dispersed in distilled water in order to produce saturated solution. The saturated solution was then mixed with soybean lecithin that was dissolved in anhydrous ethanol at β-CD:lecithin molar ratio of 0.5:1, 1:1, 2:1 and 3:1, respectively. The β-CD/lecithin mixture solution was further stirred at temperature of 60^{o}C for 2 h. It was followed by storage at 4^{o}C for 24 h. The precipitated β-CD/lecithin complex was finally obtained by centrifugation. The obtained precipitate was further washed with anhydrous ethanol to remove lecithin absorbed on the surface followed by drying in a vacuum oven until its weight reached constant.

1.2 Characterization

i. Differential scanning calorimetry (DSC): Thermal analyses of lecithin, β-CD and the β-CD/lecithin complex were performed by a Diamond DSC (Q20).

*Corresponding author: hatijah@uthm.edu.my

penerbit.uthm.edu.my/ojs/index.php/ijie

These samples were studied by heating samples in aluminum hermetic pans (Tzero, Switzerland) at a heating rate of 10ºC/min from 0 to 260-300 ºC under ultrahigh-purity nitrogen atmosphere [4].

ii. Fourier-transform infrared spectroscopy (FTIR): The FTIR spectra of lecithin, β-CD and the β-CD/lecithin complex were recorded on a UATR Two spectrophotometer (PerkinElmer). 16 scans were taken for each of them at a wavenumber recorded from 4000 to 450 cm^{-1} with a resolution of 4 cm^{-1} in the transmission mode.

iii. Scanning electron microscope (SEM): SEM analysis was performed using Phenom ProX desktop scanning electron microscope (Phenom World) at different magnification. Prior to analysis, the samples were first placed on the specimen stub using double adhesive tape. The micrographs were taken with an accelerating potential of 10 kV under low vacuum.

iv. DPPH radical scavenging assay: 2 mL of DPPH solution (0.2 mmol/L in ethanol) was incubated with 2 mL of ascorbic acid, β-CD, lecithin and β-CD/lecithin complex. The reaction mixture was shaken and incubated in the dark for 30 min at room temperature. The absorbance was then read at 517 nm against ethanol. Controls containing ethanol instead of the antioxidant solution and blanks containing ethanol instead of DPPH solution were also made. The inhibition of the DPPH radical by the samples was calculated according to the following formula:

$$DPPH\ scavenging\ activity = \frac{Abs.of\ control - (Abs.of\ sample - Abs.of\ blank)}{Abs.of\ control} \times 100\% \quad (2)$$

2. Results and Discussion

From the DSC results (**Table 1**), lecithin exhibits two endothermic peaks at 130.1ºC (deep peak) and 195.2ºC (small peak), respectively. The first peak attributes to the fusion of its crystalline portion while the second peak corresponds to the decomposition of lecithin. β-CD meanwhile exhibits a deep peak at 183.5 ºC and small peak at 312.1ºC. The occurrence of the first peak could be due to the occurrence of water releasing while the second peak refers to the phase transition of β-CD.

Table 1: DSC results of β-CD, soybean lecithin and β-CD/soybean lecithin complexes.

Sample	Weight (mg)	Endothermic peaks (ºC)
lecithin	10.64	130.1; 195.2
β-CD	7.85	183.5; 312.1
β-CD/lecithin complex (0.5:1)	7.46	175.8
β-CD/lecithin complex (1:1)	11.77	174.5
β-CD/lecithin complex (2:1)	7.06	177.8
β-CD/lecithin complex (3:1)	9.11	148.8; 158.6

As can be seen, all complexes exhibit higher melting point compared to the lecithin. The complex made of β-CD:lecithin ratio of 0.5:1, 1:1, 2:1 and 3:1, shows the melting point at 175.8ºC, 174.5ºC, 177.8ºC and 148.8 ºC/158.6ºC, respectively. The melting points of the complexes of ratios 0.5:1, 1:1 and 2:1 are slightly lower than that of β-CD while for complex of ratio 3:1, its melting point is found to increase slightly compared to the rest of complexes. Similar reduction was found by Nkanga et.al. where the addition of lecithin has shifted the endothermic peak of isoniazid from 173 °C to 130 °C [5]. Overall, this clearly shows that the thermal stabilities of all complexes were improved. Based on the results, the complex made of ratio 2:1 demonstrates the highest melting point (177.8ºC), indicating its highest thermal stability among the complexes synthesized.

FTIR is a common tool that can be used to investigate the variation of peak shape position and intensity and consequently, to confirm the formation of inclusion complex [4]. From FTIR spectroscopy results (**Figure 1**), it is found that the soybean lecithin exhibits bands at 2923.55 cm^{-1} (for C-H stretching vibration of methylene group), 1743.88 cm^{-1} (for C=O stretching vibration), 1457.51 cm^{-1} (for C-H stretching vibration of methyl group) and 1079.96 cm^{-1} (for P-O-C stretching vibration). β-CD meanwhile exhibits broad band with a transmittance peak at 3272.23 cm^{-1}, indicating the symmetric and asymmetric O-H stretching vibration. The presence of other bands at 2933.10 cm^{-1}, 1648.60 cm^{-1}, 1121.70 cm^{-1} and 1018.60 cm^{-1} in the β-CD can be attributed to C-H stretching vibration; H-O-H bending; asymmetric C-O-C stretching vibration and symmetric C-O-C stretching vibration, respectively. Results are similar to the peaks of soy-lecithin obtained by Shah et. al. [6]. In the previous study of Shah and co-workers, the addition of 0.2% lecithin dosage was found to influence the performance of compression ignition (CI) engine.

For complex made of ratio 0.5:1, two bands (1647.45 cm^{-1} and 1022.73 cm^{-1}) that are similar to that of β-CD are observed. The presence of other bands meanwhile is due to the lecithin. Similar results are also reported for complexes made of ratios 1:1 and 2:1. However, for complex made of highest β-CD content (ratio 2:1), the band at 1743.88 cm^{-1} which is due to the lecithin is not found. This suggests that the particular band is enclosed by the β-CD. For complex made of ratio 3:1, all bands are almost similar to that of β-CD. This indicates that all absorption bands of lecithin are covered by that of β-CD and that the lecithin has entered fully into the cavity of β-CD, leading to the formation of β-CD/lecithin complex.

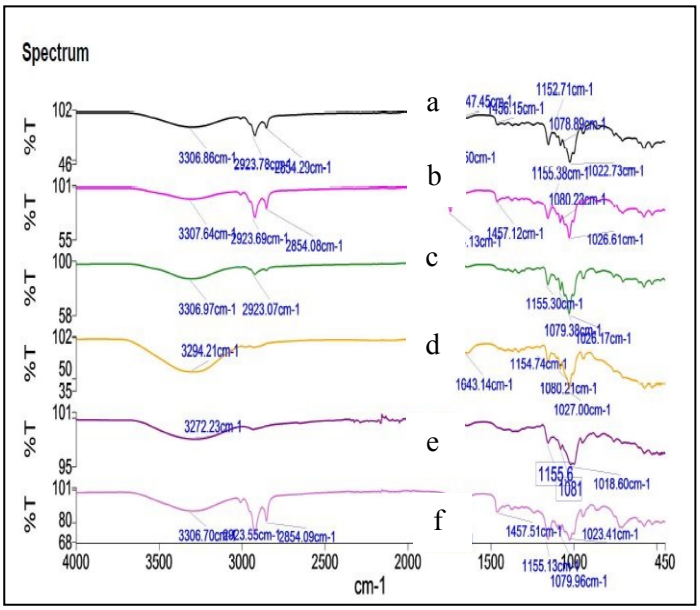

Figure 1: FTIR spectra of β-CD/lecithin complexes of ratio (a) 0.5:1; (b) 1:1; (c) 2:1; (d) 3:1; (e) β-CD and (f) lecithin.

The SEM micrographs clearly show the difference between the β-CD and the β-CD/lecithin complex, (**Figure 2**). β-CD is observed as plate shaped crystals with relatively smooth surface. After the complexation, its morphology changes dramatically in which its surface becomes very rough with irregular agglomerated shape. This phenomenon is probably due to the fact that the regular arrangement of β-CD is disrupted by the lecithin molecules with some of its parts such as the C=O bonds being encapsulated in the cavity of β-CD and the other parts of lecithin lying outside the cavity.

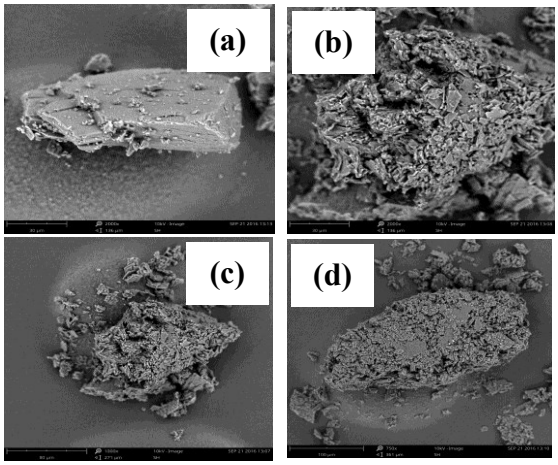

Figure 2: SEM of (a) β-CD (2000x) and β-CD/lecithin complex of ratio 2:1 at magnification of (b) 2000x; (c) 1000x; (d) 750x

Overall, the size and shape of complex are significantly different compared to the pure β-CD in which the actual morphology of the pure β-CD disappeared. The changes are the strong indication of the formation of complex by molecular encapsulation [7]. SEM images of β-CD/lecithin complex were also found similar to the super-critical anti-solvent (SAS) encapsulated lycopene with lecithin observed in Cheng et.al. [8]. In their study, Cheng et.al. has proved that SAS encapsulation of lycopene with lecithin and α-tocopherol has enhanced the stability of lycopene.

Table 2 has shown the DPPH scavenging activity of soybean lecithin and β-CD. It has been colorimetrically measured based on the reduction of diphenylpicrylhydrazine (DPPH) radical from violet to yellow color when the stable DPPH radical accepts an electron from the antioxidant compound [9]. Complex made of ratio of 2:1 exhibit the highest activity (97.10%) followed by the complex made of ratio of 0.5:1 (94.46%), ratio of 1:1 (86.93%) and ratio of 3:1 (63.04%). The complexes show an increase of radical scavenging activity in the order C2:1 > C0.5:1 > C1:1 > C3:1. Standards and all the complexes showed a dose dependent inhibition of the DPPH radicals. The results clearly indicate that the complex made of ratio of 2:1 has the greatest enhancement with respect to antioxidant property in comparison to the original soybean lecithin.

Table 2: DPPH scavenging activity (%) of all the samples tested.

Sample	Abs				DPPH scavenging activity (%)
	1st	2nd	3rd	Average value	
lecithin	1.237	1.219	1.298	1.251	14.65
β-CD	0.010	0.007	0.004	0.007	96.77
C 0.5:1	0.042	0.042	0.042	0.042	94.46
C 1:1	0.203	0.136	0.129	0.156	86.93
C 2:1	0.001	0.003	0.003	0.002	97.10
C 3:1	0.566	0.505	0.484	0.518	63.04
Ascorbic acid	0.303	0.300	0.297	0.300	77.43
Control	1.522	1.519	1.505	1.515	-
Blank	0.042	0.042	0.042	0.042	-

3. Summary

In summary, upon the complex formation, all complexes exhibited increased thermal stability and antioxidant property. The results of FTIR and SEM have confirmed on the β-CD/lecithin complex formation. From the overall results, it was found that the best β-CD/lecithin ratio was recorded at 2:1 as the complex exhibited the greatest thermal stability coupled with highest melting point. Furthermore, it also displayed the greatest antioxidant property as indicated by its highest percentage of DPPH scavenging activity.

References

[1] L. Szente, and J. Szejtli, "Cyclodextrins as food ingredients," Journal of Food Science & Technology, vol. 15, pp. 137–142, 2003.

[2] J. Surh, Y. Jeong, and G. Vladisavljevic, "On the preparation of lecithin-stabilized oil-in-water emulsions by multi-stage premix membrane

emulsification," Journal of Food Engineering, vol. 89, pp. 164–170, 2008.
[3] Y. Wu, and T. Wang, "Fractionation of crude soybean lecithin with aqueous ethanol," Journal of the American Oil Chemists' Society, vol. 81, pp. 697–704, 2004.
[4] X. Wang, Z. Luo, and Z. Xiao, "Preparation, characterization, and thermal stability of β-cyclodextrin/soybean lecithin inclusion complex," Carbohydrate Polymers, 101, 1027–1032, 2014.
[5] C.I. Nkanga, R.W. Krause, X.S. Noundou, R.B. Walker, "Preparation and Characterization of isoniazid-loaded Crude Soybean Lecithin Liposomes", International Journal of Pharmaceutics, 526, 466-473, 2017.
[6] P.R. Shah, U.N. Gaitonde, A. Ganesh, "Influence of soy-lecithin as bio-additive with straight vegetable oil on CI engine characteristics", Renewable Energy, 115, 685-696, 2018.
[7] N. Rajendiran, and S. Siva, "Inclusion complex of sulfadimethoxine with cyclodextrins: Preparation and characterization," Carbohydrate Polymers, 101, 828– 836, 2013.
[8] Y-S. Cheng, P-M. Lu, C-Y. Huang, J-J. Wu, "Encapsulation of lycopene with lecithin and α-tocopherol by supercritical antisolvent process for stability enhancement", The Journal of Supercritical Fluids, 130, 246-252, 2107.
[9] K. Pavithra and S. Vadivukkarasi, "Evaluation of Free Radical Scavanging Activity of Various Extracts of Leaves from *Kedrostis foetidissima* (Jacq.) Cogn.", Food Science and Human Wellness, 4, 42-46, 2015.