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MICROENCAPSULATION BY FREEZE DRYING OF LIMONIN USING β-CYCLODEXTRIN AND ITS STABILITY IN DIFFERENT pH SOLUTION

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Abstract

The study aims to determine the effect of limonin core to coating ratio on limonin microencapsulation efficiency and its stability in pH 4 and 7 solution. Encapsulant used was β-cyclodextrin with core to coating ratio 1:10 and 1:20. Microencapsulation process was conducted using freeze dryer. In the analysis, parameters were tested (including encapsulation efficiency) and characterized using a spectrophotometer. Stability of limonin microcapsule in pH 4 and 7 solution was conducted for 12 days. Limonin microencapsulate surface was observed using a scanning electron microscope. Experimental results based on electron microscope showed that Limonin microcapsule has irregular shape and size. Results of the study also revealed that limonin microencapsulation with core to coating ratio of 1:10 had efficiency of 68.14 %, while that with ratio of 1:20 had efficiency of 80.52%.

Keywords: β-cyclodextrin, Freeze drying, Limonin, Microencapsulation, pH, Stability pH.

1. Introduction

Citrus belongs to the family Rutaceae is a source for bioactive compound [1]. Citrus contains phytochemicals such as carotenoids, flavonoid and folate and with the vitamin C content, citrus contribute to cancer prevention [2]. Isolated 39 limonoid aglycone [3] and 21 limonoid glucoside from citrus and limonin and nomilin are the major citrus bioflavanoid that is the most dominant in citrus fruit [4]. Limonin, also known as limonoate d-ring lacton, is a major bioflavanoid in citrus that is also responsible for delayed bitterness [5] and concentrated in the citrus seed [6]. Limonin showed defensive role on detoxification system in aflatoxin B1 in Wistar albino rat therefore limonin has excellent anticancer activity [7]. Limonin also has excellent antioxidant capacity by supressing lipid peroxidation and defending chemically induced liver damage on rat [8].

Healthy or functional food often contains phytochemical that has bitter taste. Thus, it is important to reduce bitterness due to consumer sensory perception [9]. Oral consumption or addition of limonin to food system could be a problem due to the bitter taste. Bitter taste can be masked by the application of encapsulation using polymer, cyclodextrin, lipid, or surfactant [9]. Microencapsulation does not only help masking bitterness of bioactive compound but also help protect them from damages caused by oxygen, heat, or light [10]. The effectiveness of phytochemical compound antioxidant activity depends on bioactivity, stability, and bioavailability of these compound, which could be overcome with encapsulation technology [11]. Microencapsulation of bioactive compound from red grape can reduce bitterness as well as stabilize polyphenols content [12]. Microencapsulation process commonly uses spray dryer or freeze dryer. Bioavailability of bioactive compound and organoleptic characteristics in freeze dried microencapsulated product were better since the minimum use of heating compared with spray dried microencapsulated product [13]. Application of microencapsulation offers lycopene in better stability against heat and oxygen. Then, it is free from releasing homogenous pigment in the food system [14]. The use of freeze dried microencapsulation of blueberry juice using cyclodextrin showed better retention values of anthocyanin and very high radical scavenging activity compared with spray-dried microencapsulated blueberry juice [15].

β-cyclodextrin is one of the most common materials used in microencapsulation process. β-cyclodextrin comprises seven units of glucopyranose consider a GRAS (general recognize as safe by FDA [16]. Application of 0.5% β-cyclodextrin reduce bitterness in citrus juice since β-cyclodextrin made complex with limonin [17]. Limonin bitterness can be reduced by the use of β-Cyclodextrin with efficiency of 94% [18]. β-cyclodextrin has the ability to reduce bitterness due to their hydrophobic cavity and exterior shell where limonin interact with the interior part of cyclodextrin forming inclusion complex thus unable to bind Taste receptor cells [19]. β-cyclodextrin form inclusion complex with other molecule by non-covalent bond and complex stability becomes better with the availability of electron-donor character of the substituents [16]. β-cyclodextrin can make a stable complex from with the guest molecule that is less polar than water or less hydrophilic [9]. The use of β-cyclodextrin was expected to give limonin stability against light, pH, and heat, as well as mask their bitterness when added to food system or consumed by consumer.

Core to coating ratio can effect encapsulation efficiency. Microencapsulation of Avverhoa carambola using maltodextrin shows the highest efficiency resulted from core to coating ration 1: 20 [20]. The use of β-cyclodextrin core to coating ratio, which is 1: 6, resulted in encapsulation efficiency of 78% [15]. β-cyclodextrin can make inclusion complex without solvent availability but the presence of water is needed for the process [9]. Therefore, 10% β-cyclodextrin solution was made for microencapsulation process.

The study aims to determine the effect of core to coating ratio on microencapsulation efficiency of limonin and determine stability of limonin microcapsules in pH 4 and 7 solution at 12 days storage in room temperature. β-cyclodextrin in 10% concentration was used as coating material.

2. Material and Methods

2.1. Material and reagents

Citrus seeds are a waste material obtained from citrus juice sellers around university. Reagents for limonin extraction were n-hexane, acetone, dichloromethane, isopropanol. Reagents for limonin extraction were technical grade and obtained from Brataco chemicals, Indonesia. Reagent for limonin analysis were ethanol, 4-dimethylamino benzaldehide (sigma aldrich, USA), acetic acid glacial, perchloric acid, chloroform, limonin standards, and acetonitrile. Reagents for limonin analysis were pro analysis grade and obtained from Merck and Smart Lab Chemical, Indonesia. Material used for encapsulation was β-cyclodextrin and obtained from Sigma aldrich, USA. Solution pH 4 and 7 were made by adding citric acid monohydrate and disodium hydrogen phosphate to distilled water. All chemicals used for making pH 4 and 7 solution were pro analysis grade and obtained from Merck, Germany.

The equipment used in this study were heating mantle (Huang Hua, China), soxhlet extraction devices (Isolab, Germany), vortex mixer (Ika, Germany), spectrophotometer (Mapada, Japan), sentrifugator (Nesco, China), rotary vacuum evaporator (Ika, Germany), oven (Etuves, France), analytical balance (Mettler Toledo, USA), freeze dryer (Eyela, Japan), and glasses apparatus (Isolab, Germany).

2.1.1. Limonin extraction

Limonin extraction conducted based on extraction using n-hexane and acetone respectively [21]. Citrus seeds were dried in an oven until the weight was constant and grounded. Limonin extract was analyzed to determine total limonin content using method based on reaction of furan wing with burham reagent in acid condition and its absorbance was measured using spectrophotometer at wavelength of 503 nm [22].

2.1.2. Production of microcapsules

Encapsulate was made by dissolving 10 grams of β-cyclodextrin in 100 ml distilled water and was continuously mixed using hot plate magnetic stirrer. Limonin then added by ratio of 1:10 and 1: 20. Limonin microencapsulation used freeze dryer with condition pressure < 0,01 mmHg (< 133 Pa) and temperature of between -40 and 20°C for 24 hours. Microcapsules were weigh and stored in desiccator.

2.1.3. Encapsulation efficiency

Encapsulation efficiency was calculated by determining ration of limonin content in capsule surface and total limonin content.

2.1.4. Analysis of microcapsule surface using SEM

The microcapsule surface was analysed by scanning electron microscope (SEM) using JSM-6510 (JEOL, Japan). The samples were placed on metallic adhesive tape. SEM images were taken at 3.000 and 10.000 x magnification. The observations were made at voltage at 10 kV.

2.2. Solvent residue analysis

Solvent residue analysis was conducted to determine whether organic solvent used in limonin extraction exist in limonin microcapsule. Determination of residue solvent carried out with GC-MS (Shimadzu, Japan) by dissolving microcapsules with solvent that miscible with solvents used for limonin extraction [23].

2.3. Limonin stability on aqueous pH 4 and pH 7

pH 4 solution made with citric acid monohydrate in distilled water pH 7 solution was made with disodium hydrogen phosphate in distilled water. Limonin microcapsule was added to pH 4 and 7 solution at concentration of 2% and stored in glass bottle made at in dark room temperature. Limonin concentration on solution was measured every 2 days for 12 days. The measurement of limonin concentration on pH solution based on method proposed by literature [24].

3. Results and Discussion

3.1. Scanning Electron Microscope (SEM) analysis of limonin microcapsule

Irregular size and uneven structure were found in β-cyclodextrin at 2300 and 100,000 × magnification (See Figs. 1 and 2). This is in a good agreement with reference [25]. Scanning electron images of limonin microcapsule prepared from β-cyclodextrin with core to coating ratio 1: 10 showed irregular shape as seen in Fig. 3. Variation in microcapsule morphology such as structure and size, might be due to thermal expansion inside drying particles, pressure and external fracture [26]. Phenolic extract encapsulated with b cyclodextrin showed irregular shaped particles obtained from lyophilization characteristic [27]. Core to coating ratio did not affected the shape of limonin microcapsules.

β-cyclodextrin particles have irregular shape, when limonin and β -cyclodetxrin mixed together, they continue to exist in original shape. Microencapsulation of cathechin with core to coating ratio 1: 1 showed β-cyclodextrin particles were tiny aggregates of irregular size 5 - 100 um, while morphology of limonin microencapsulated with core to coating showed most of the particles were needle like with size of 5 um [28].

Limonin crystal size is crystal size 0.28 × 0.22 × 0.15 um [29]. While SEM analysis of limonin microcapsule showed irregular shape and size. Change in

morphology and shape particle might happen during freeze drying revealing interaction between cyclodextrin and limonin [30].

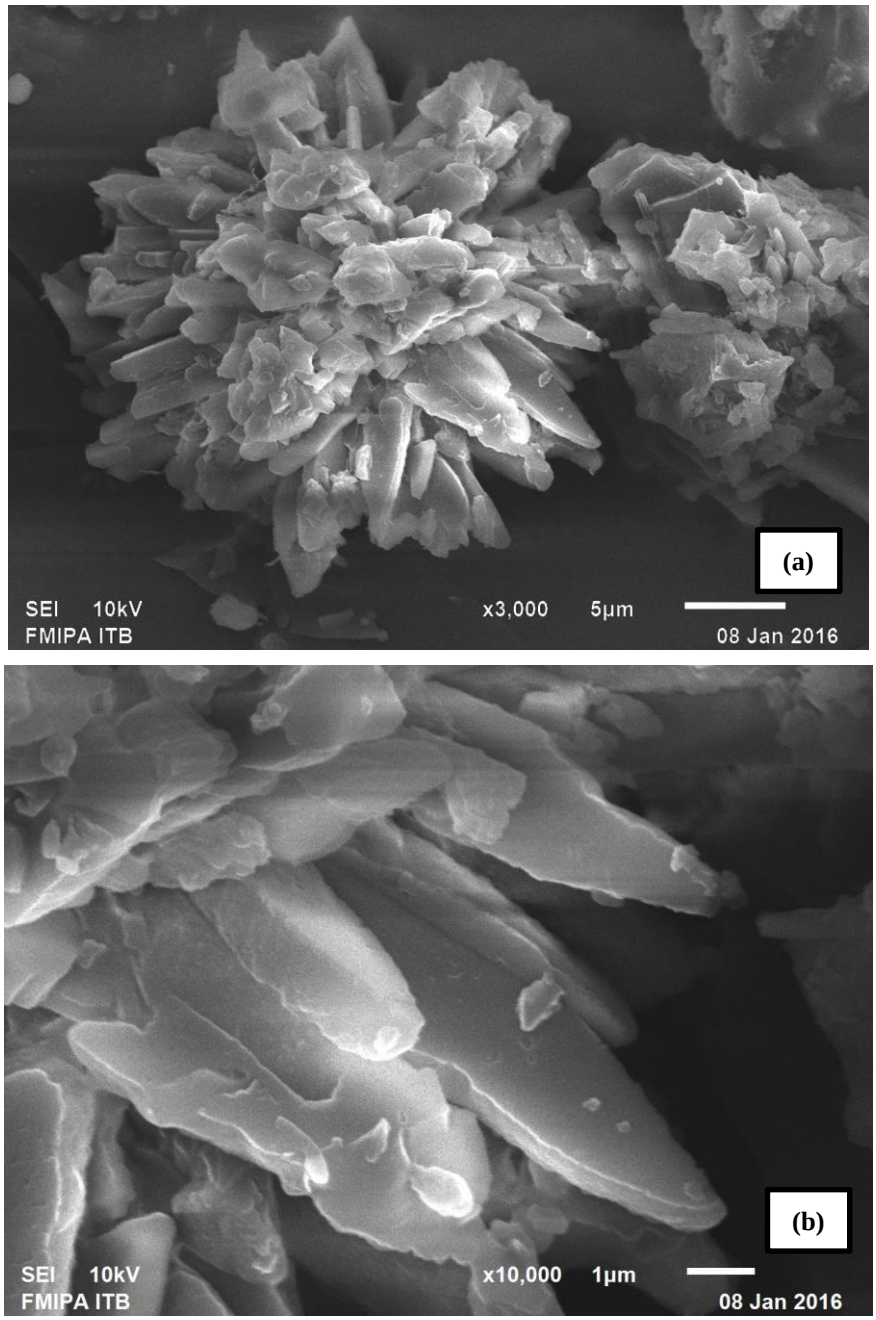


Fig. 1. SEM Images of limonin microcapsules (a) with core to coating material ratio of 1: 10 at 3000 x magnitude; (b) with core to coating material ratio of 1: 10 at 10.000 × magnitude.

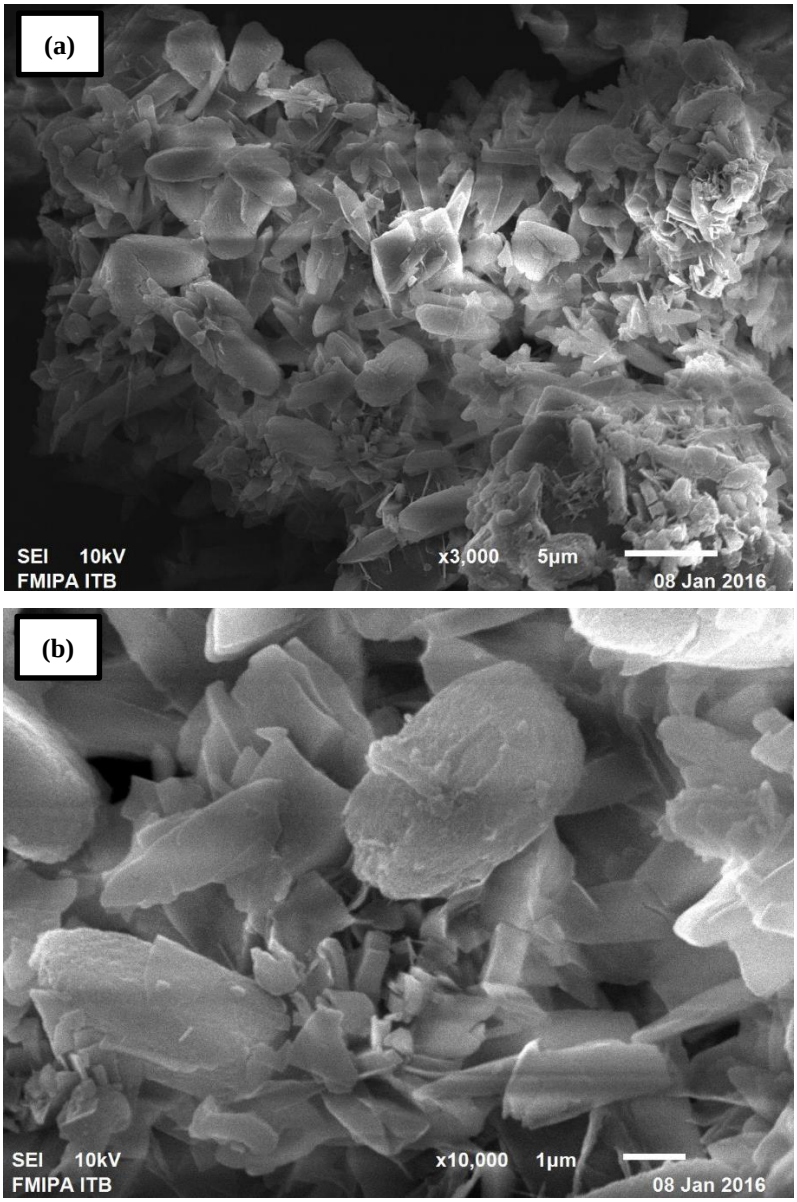


Fig. 2. SEM Images of limonin microcapsules (a) with core to coating material ratio of 1: 20 at 3000 x magnitude; (b) with core to coating material ratio of 1: 20 at 10.000 × magnitude.

3.2. Efficiency of encapsulation

Total limonin content in precipitate and microcapsule were determined by the method described in Material and Method section. Total limonin content of precipitate was 1.213 mg and total limonin content on cyclodextrin inclusion complex with core to coating ratio of 1 : 10 dan 1 : 20 were determined as 0.386 mg and 0.236 mg, respectively.

Encapsulation efficiency was based on the amount of limonin in capsule surface compared with limonin concentration. Lower concentration of limonin measured after encapsulation might due to entrapment of limonin in the β-cyclodextrin. There was no significant difference ($p \leq 0,05$) between core and coating ratio 1: 10 and 1: 20 on encapsulation efficiency. Encapsulation efficiency of microcapsules with core to coating ratio 1: 10 and 1: 20 were 68,14%% and 80,52% respectively. The result is higher, compared with encapsulation efficiency of phenolic extract of blackberry using β-cyclodextrin with core to coating ratio 1: 1, which was 54% [31]. Freeze method of curcumin encapsulation in cyclodextrin with core to coating ratio 1: 1 resulted in efficiency of 22.8% [32]. Freeze-drying method of bell pepper extract encapsulation using β -cyclodextrin with core to coating ratio 1: 4 was 40.79% [33]. Encapsulation efficiency of cathechin in β -cyclodextrin with core to coating ratio 1: 6 was 94%. Efficiency encapsulation of essential oil in β -cyclodextrin with freeze-drying method range from 41.7 to 84.7% [34].

Cyclodextrin is an amphyphilic molecule where inner face is hydrophobic and outer face is hydrophilic with inner diameter of 5-10 A. In solution, hydrophilic outer part is hydrated but hydrophobic inner face form thermodynamically stable complex [28]. Encapsulation efficiency is affected by both types of capsules and core components. It also depends on purity of the core compound. Other researchers showed pure compound has higher efficiency compare with extract [34].

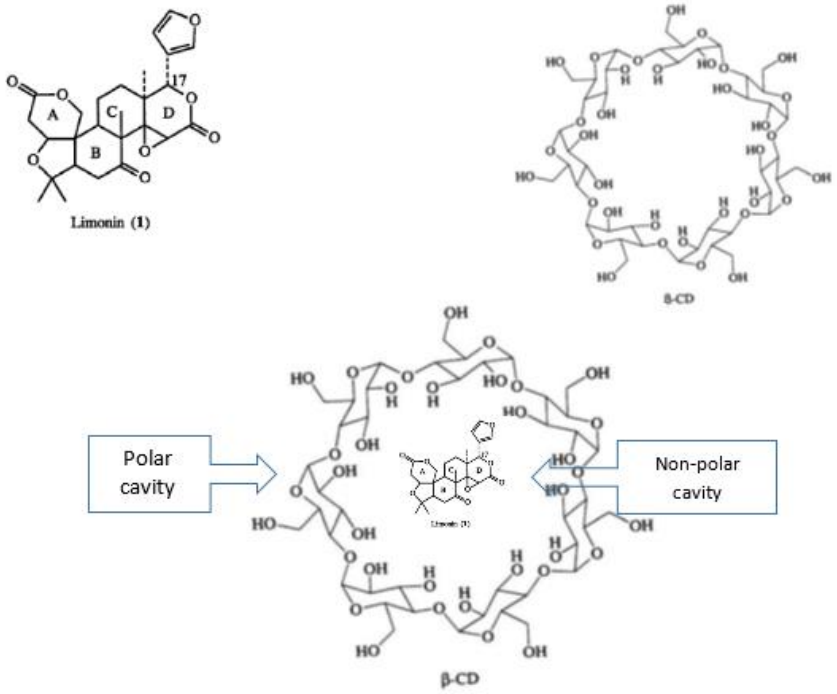


Fig. 3. (a) Limonin Structure (Hasegawa et al, 1997) ; (b) β-cyclodextrin structure [16]; (c) inclusion complex between limonin and β-cyclodextrin.

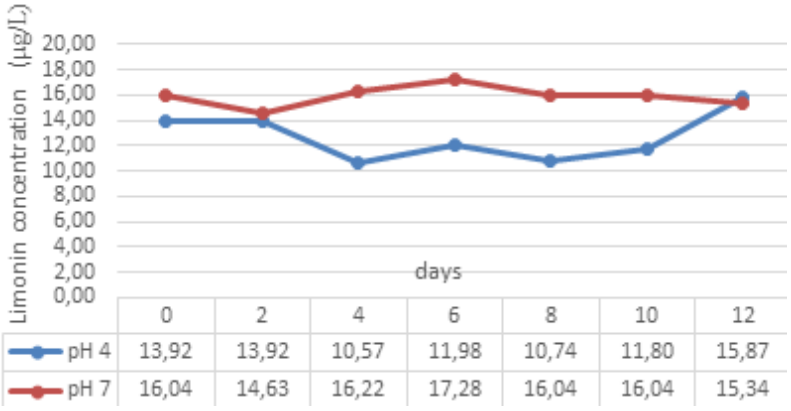
3.3. Solvent residue analysis

Solvent residue analysis carried out to make sure no solvent detected in limonin microcapsules therefore it will be safe for application on food system. Solvent residue analysis [35] uses Gas Chromatography-Mass Spectrophotometer equipment. The solvent used was chloroform since it has the same miscibility with dichloromethane, hexane and isopropyl alcohol used in limonin extraction. The result show no organic solvent detected in limonin microcapsules.

3.4. Stability of limonin microcapsules in pH solution

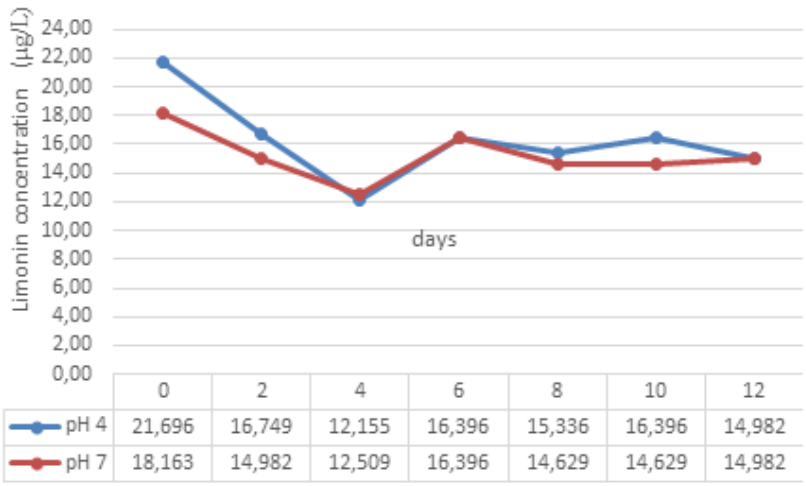
Encapsulated phytochemical compound possesses stability since it is isolated from the environment (See Figs. 4 and 5). Although there are several factors that a trigger core release which are pH, temperature and pressure [31]. When flavonoid or phenolic compound encapsulated with cyclodextrin and form complex inclusion, the antioxidant and antibacterial properties could be affected and rates of antioxidant degradation could be decreased encapsulated flavonoid can be release under acidic pH or increase temperature [36].

During storage of limonin microcapsule in pH 4 and 7 solution, release of limonin into solution remains low. Fluctuation of limonin concentration in the solution might due to limonin release from microcapsule surface and remain in the solution since limonin stable in acidic condition [37]. Limonin microcapsules in pH 7 solution yielded low release concentration compared with pH 4. This behaviour might be due to the vulnerability of capsules to be broken in low pH because of higher solubility of wall material. Blackberry extract encapsulated with β -cyclodextrin also show the same pattern [31]. Storage of beverage contain limonin glucoside showed limonin decreased 42% and 37% under storage condition of room temperature and glass-door refrigerator respectively [38]. Application of bell pepper extract microcapsule in yoghurt showed higher stability in coloured within 10 days compared with bell pepper extract. Complex inclusion of cathecin in β -cyclodextrin improved water solubility and protect cathechin against temperature, light, oxygen therefore prolonge storage stability [28].



	0	2	4	6	8	10	12
pH 4	13,92	13,92	10,57	11,98	10,74	11,80	15,87
pH 7	16,04	14,63	16,22	17,28	16,04	16,04	15,34

Fig. 4. Stability of β-Cyclodextrin Limonin microencapsulated ratio 1: 10 in pH 4 and pH 7 Solution.



	0	2	4	6	8	10	12
pH 4	21,696	16,749	12,155	16,396	15,336	16,396	14,982
pH 7	18,163	14,982	12,509	16,396	14,629	14,629	14,982

Fig. 5. Stability of β-Cyclodextrin Limonin microencapsulated ratio 1: 20 in pH 4 and pH 7 Solution.

4. Conclusion

The results of the study reveal different limonin core to coating ratio causing different encapsulation efficiency. Encapsulation efficiency of limonin microcapsules with core to coating ratio 1: 10 and 1: 20 were 68.14% and 80,52% respectively. Morphology of microcapsules surface showed irregular shape and size. Limonin microcapsule was stable during 12 days of storage at 27°C and only 2% of limonin released into solution. Limonin microcapsules in pH 7 solution yielded low release concentration compared with pH 4 solution.

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