Microencapsulation of Limonin From Orange Juice Waste Using Maltodextrin

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Abstract. The study aims were to determine the effect of ratio between limonin and encapsulant on encapsulation efficiency and to know limonin encapsulant stability on aqueous with pH value of 7. Research was conducted using descriptive method and limonin stability was measured every 2 days for 14 days using spectrofotometer methods. Aqueous with pH value of 7 was made by adding disodium hydrogen phosphate to aquades until pH value reached 7. Results showed ratio on limonin versus maltodextrin 1: 10, had encapsulation efficiency of 89.73%. Maltodextrin had a straight chain so that the material was more easily enter into encapsulant. Limonin was stable on acidic aqueous with pH value 5-7. Application on limonin encapsulation on aqueous with pH value of 7, showed that limonin activity had two peak on day 4 and 12. Limonin encapsulant had specific pattern of time release that help it to be easier for application on food or medicine.

1. Introduction

Limonin had high value amounted US $ 109.5 to 5 mg of pure limonin (SIGMA, 2009). Limonin is the main limonoids compounds found in almost types of oranges and is the main cause of a bitter taste in the orange juice. Nevertheless, according to [2], limonin has great benefits for health because it can increase the activity of the enzyme glutathione S-transferase thus inhibiting tumor growth, whereas according to [3]; [4], limonin helps lower cholesterol ratio of LDL / HDL and accelerates the oxidation of low density lipoprotein (LDL) in the intestines, helping to reduce risk of deep vein thrombosis.

Limonin (Limonic Acid 3.19: 16, 17-dilactone) is limonoids aglycone compound with the molecular formula: C₂₆H₃₀O₈ molecular weight 470.5 Da, the boiling point of 280 ° C, is insoluble in water, but soluble in dimethylformamide, dichloromethane, acetonitrile, glacial acetic acid, and alcohol [5]. It is a yellowish-white crystalline form, found in all citrus species, with the highest content in citrus seeds. Limonin in citrus fruits initially contained as limonoic acid compound A-ring lactone which has a lactone group and does not have a bitter taste, when the orange juice extraction processed, these compounds lactonized become limonoids dilakton which has two lactone group and have a bitter taste [6]. This process is catalyzed by acid and influenced by enzyme activity. Exposure to the heat during pasteurization and evaporation will accelerate the reaction [7].

Limonin isolation as the use of orange juice by-products is expected to give added value to the orange juice industry. Based on food technology perspective, using powder form of bioactive compounds is easily applied to food product. Microencapsulation limonin in this research has been conducted malodekstrin as encapsulan. Microencapsulation process engineering using a freeze dryer. The study aims were to determine the effect of ratio between limonin and encapsulant on encapsulation efficiency of 89.73%.
efficiency and to know limonin encapsulant stability on aqueous with pH value of 7. Aqueous with pH value of 7 was made by adding disodium hydrogen phosphate to aquades until pH of 7. Research was conducted using descriptive method and limonin stability was measured every 2 days for 14 days using spectrophotometer methods proposed by [8] modified by [9].

2. Methodology

1.1 Time and Place
Research carried out for 16 months, from March 2015 to August 2016, in the Laboratory of Agricultural Product Processing Technology Education Program and laboratory of Quality Control, Laboratorium of chemical instrument and the Laboratory of Industrial Technology, Faculty of Agricultural Technology IPB.

3. Materials and Methods
Materials used in this study is lime, chemicals for extraction limonin consisting of hexane, acetone, dichloromethane, isopropanol, chemicals for analysis consisting of: ethanol, acetone, isopropanol, hexane, dichloromethane, 4-dimethylamino benzaldehyde, acid Glacial acetic, perchloric acid, chloroform, methanol, limonin standards, and acetonitrile, cyclodextrin, maltodextrin.
The equipment used in this study are heating mantle, vortex mixer, spectrophotometer, sentrifugator, rotary vacuum evaporator, oven, analytical balance, separating funnel, freeze dryer, Soxhlet extraction devices and tools glasses.

4. Research procedure

1.2 Preparation of Raw Materials
The raw materials used for the isolation of limonin is an orange seed because it is the most widely-containing limonin. To simplify the process of extraction, citrus seeds need to be dried using an oven temperature of 50 °C up to grapefruit seed weight stable. Furthermore grapefruit seed crushed to increase the surface area so that the greater the surface area exposed to the more efficient solvent extraction process.

1.3 Isolation extracting limonin with several solvents
Limonin isolation by extraction soxhletasi conducted in several stages, the first stage using hexane, which aims to remove the oil and non-polar components of grapefruit, the second stage using acetone which aims to extract limonin using Soxhlet extraction device. Limonin isolation process diagram of seeds is presented in Figure 1.
Orange seeds

Drying $T \, 50^\circ C, \, t = 24$ hours

Grinding

Dried orange seed

Soxhletation extraction using n-hexan

Soxhletation extraction using acetone

Evaporation

Acetone extract

Precipitation using hexan with ratio $1:3$ for 16 hours

Precipitates

Purification using dichloromethane and isopropanol with ratio $1:4$ for 20 hours

Limonin crystal

**Figure 1.** Limonin Extraction
1.4 Preparation of microcapsules  
Coating for microencapsulation process is made of cyclodextrin and maltodextrin. Coating is made by dissolving the cyclodextrin using distilled water at a concentration of 10% and heated at 50°C while stirring using a magnetic stirrer hot plate. The suspension then added powdere limonin in some comparisons.

1.5 Microencapsulation limonin  
Microencapsulation process is performed using a freeze in which a suspension containing limonin and enkapsulan dibekuan for 12 hours subsequent freeze drying for 24 hours. Once completed, powder drying results are weighed and stored in a desiccator at room temperature.

1.6 Measurement of Total limonin content  
Limonin content measurement refers to the method Abbasi et al (2005) crystal reacting limonin is extracted with Burham reagent and absorbance was measured using a spectrophotometer at a wavelength of 503 nm.

1.7 Measurement of surface limonin  
Limonin content measurement surface refers to methods Saenz et al modified. Limonin levels on the surface of the microcapsules is calculated by dissolving 100 mg of microcapsules in 1 ml of acetonitrile for 1 minute. Limonin levels on the surface are subsequently measured using the same method with a measurement of total limonin.

1.8 The encapsulation efficiency  
Encapsulation efficiency is the ratio of the concentration levels of limonin encapsulation of limonin total. Levels of limonin encapsulation is the difference in the levels of total grading limonin limonin capsule surface. Encapsulation efficiency is calculated by:

\[ E \times 100 = \left( \frac{T}{S} \right) x 1 \]

1.9 Measurement of Limonin encapsulant Stability on aqueous pH 7  
Limonin encapsulant was added to aqueous with pH value 7 and its stability was measure every 2 days for 14 days. Limonin concentration was measured according to method proposed by Abbasi et al (2005) which were reacting limonin precipitate with burham reagent (consist of 4-dimethylaminobenzaldehyde, percloric acid, acetic glacial acid) and the absorbance was measured using spectrofotometer with wavelength of 503 nm. Process diagram of limonin encapsulation and stabulity analysis on aqueous pH 7 shown in Figure 2.

5. Result and Discussion  
Encapsulation is successful if it has a high efficiency. Factors affect the encapsulation efficiency were ratio versus material and enkapsulan, equipment used and conditions of the equipment. Microencapsulation can be conducted with a spray dryer or freeze dryer. Freeze dryer advantages was for products that are sensitive to heat. At the beginning of freeze drying, the surface of the solution into a solid and amorp allow the material to diffuse into encapsulant.
Figure 2. Limonin Encapsulation and Stability Analysis on Aqueous pH 7
Results showed microencapsulation with the highest efficiency is obtained from the treatment with a type of enkapsulan maltodextrin ratio versus material enkapsulan of 1: 10. Maltodextrin is starch derivative compounds which have structures in the form of a straight chain comprising 10 glucose monomers. Maltodextrin structure is presented in Figure 3.

![Maltodextrin Structure](image1.png)

**Figure 3. Maltodextrin Structure**

While limonin is a flavonoid compound in the form of triterpenoids with several groups furan and many OH groups. Maltodextrin straight chain structure will be easier to trap limonin limonin which is bonded to the outside of the molecule maltodextrin slightly. This has resulted in a high encapsulation efficiency. Use of material ratio versus higher enkapsulan menurunkan efficiency because enkapsulan have a limit on the number limonin that can be wrapped so when used enkapsulan more, limonin will be attached on the outside enkapsulan. Limonin structure is presented in Figure 3.

![Limonin Structure](image2.png)

**Figure 4. Limonin Structure**

Limonin has many beneficial effect to human health, therefore encapsulation of limonin is expected to make addition of limonin to the food system would be easier. Since Limonin extraction was using organic solvent such as n-hexan and acetone and the precipitation process was also using organic solvent, there were n-hexan, dcloromethane and isopropil alcohol, the solvent residue test was conducted to make sure no more organic solvent was detected on limonin encapsulation. Organic solvent residue analysis was using Gas Chromatography Mass Spectrofomer (GC MS). Chloroform was used as solvent since it could dissolve all organic solvent used in limonin extraction including n-hexan, acetone, isopropil alcohol and methylene chloride. The result show there was no retention time for solvent used in limonin extraction so it can be concluded no solvent residue was detected.

According sigma aldrich, maltodextrin pH was unknown but according to [10] maltodextrin aqueous that have pH value 2-4 had microbial stability and decrease haze. According to [11], maltodextrin storage will cause syneresis and make water release from maltodextrin. While [12] said that limonin was stabil on pH value 4 to 7 and would be degraded in alkaline. Addition of limonin encapsulant in aqueous with
pH value of 7 might release limonin from encapsulant and it has its own pattern on time release as shown in Figure 5.

![Limonin Encapsulant Stability](image)

**Figure 5.** Limonin Encapsulant Stability on Aqueous pH 7

Limonin stability on aqueous with pH value 7 has its own pattern that the time release peak was on day 4 dan 12. Maltodextrin has high solubility in water so it was fathomed that when maltodestrin dissolved, some limonin was released to aqueous causing increasing in limonin concentration on day 4 dan 12. According to [12] limonin stability on pH 7 was 4 days, it was fit with the research as shown in figured 4 that limonin concentration was decrease from day 4 to 8. Limonin that release to aqueous pH 7 was low in concentration so it can be assumed that limonin encapsulation using maltodextrin was stable on pH 7 and room temperature for 14 days.

Researches conducted by [13],[14], stated that to get optimum result, encapsulant can be mixed of modified starch and protein such as carragenan, or mixed of modified starch such as maltodextrin and gum arab or CMC.

6. **Conclusions**

Ratio on limonin versus maltodetrin 1: 10, had encapsulation efficiency of 89.73%. Maltodextrin had a straight chain so that the material was more easily enter into encapsulant

Application on limonin enkapsulation on aqueous with pH value of 7, showed that limonin activity had two peak on day 4 and 12. Limonin encapsulant had spesific pattern of time release that help it to be easier for application on food or medicine.

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References

[1] SIGMA. [on-line]. http://www.sigma-aldrich.com/ [9 Feb 2007].
[2] Ishii T, Ohta H, Nogata Y, Yano M, Hasegawa S. 2003. Limonoids in Seeds of Iyo Tangor (Citrus iyo hort. ex Tanaka). Food. Sci. Technol. Res. 9 (2) : 162-164. [terhubung berkala]. http://www.jstage.jst.go.jp [27 Agst 2008].
[3] McGill, C. R., & Green, N. R. (2001). Modification of cholesterol concentrations with citrus phytochemicals. PCT Int. patent Application, WO 2001032160 A2 20010510.
[4] Dandekar DV, Jayaprakasha GJ, Patil BS. 2007. Hydrotrropic Extraction of Bioactive Limonin from Sour Orange (Citrus aurantium L.) seeds. [terhubung berkala]. http://www.sciencedirect.com [13 Nov 2008].
[5] National Center for Biotechnology Information. PubChem Compound Database; CID=179651, https://pubchem.ncbi.nlm.nih.gov/compound/179651 (accessed Oct. 19, 2016).
[6] Hasegawa, S. L.K.T Lam, E.G. Miller. 2008. Citurs Limonoids : Biochemistry And Possible Importance To Human Nutrition dalam Phytochemical and Phytopharmaecuticals. F, Shahidi dan Chi-Tang Ho (eds) (on-line) Available on-line at http://www.google.com (diakses tanggal 19 November 2008)
[7] Mozaffar Z, Mirdana QR. dan Saxena V.penemu; Sepragen Corporation. 4 Apr 2000. High Throughput Debittering. US patent 6 045 842.
[8] Abbasi S, Zdani P, dan Mirbagheri E. 2005. Quantification of limonin in Iranian Orange Juice Concentrate using High-performance Liquid Chromatography dan Spectrophotometric Methods. Eur. Food. Res. Technol 221:202-207.
[9] Setyadjit. 2005. Methods for Contaminant Analysis and Purification of Single Strength Citrus Juice. Final Report. Agency for Agriculture Research and Development. Univ Queensland.
[10] Bennet, CJ., Hazlet NJ. 1986. Method of Preparing An Aqueous Maltodextrin Solution Haivng Microbial Stability and Decrease Haze. (on-line) at http://www.google.com/patents/US4596602
[11] Chronakis, Ioannis S. 1998., Compositional Properties , and Structural-Functional Mechanisms of Maltodextrins : A Review On the Molecular Characteristics , Compositional Properties , and Structural- Functional Mechanisms of Maltodextrins : A Review. Critical Reviews in Food Science and Nutrition On the Molecular Characteristics 38(7):599–637 (on line) . [19 Oktober 2016]
[12] Jitpukdeeboontra S, Chantachum S, Ratanaphan A, Chantrapromma K. 2005. Stability of Limonin From Lime Seeds. EJEAFChe, 4 (3) : 938-944. [terhubung berkala]. http://www.ejeafche.uvigo.es [19 Mar 2009].
[13] Rivera, T, J Crouse, PS Given, jr. 2010. Microencapsulated citrus phytochemicals and application to beverages. [terhubung berkala] http://www.google.com/patents/WO2010090975A1?cl=en [10 Desember 2013]
[14] Cilek, B. A Luca, V Hasirci, S. Sapin, G Sumnu. 2012. Microencapsulation of phenolic compounds extracted from sour cherry pomace : effect of formulation, ultrasonication time and core to coating ratio. Eur Food Res technol. 235 : 587-596 [terhubung berkala]. http://www.springer.com [10 Desember 2013].