Morfology and Stability of Microencapsulation of Limonin using Maltodestrin: Morfology and Stability

D Cakrawati 1*, M N Handayani 1, E Noor 2, T C Sunarti 2

1 Study Program of Agro-industry Technology Education, Fakultas Pendidikan dan Teknologi dan Kejuruan, Universitas Pendidikan Indonesia, Jalan Dr. Setiabudhi Nomor 229 Bandung 40154, Bandung

2 Fakultas Teknologi Pertanian, Institut Pertanian Bogor, Bogor, Indonesia

*dewicakrawati@upi.edu

Abstract. The objective of the research was to study the effect of limonin core to coating ratio on efficiency limonin and stability of limonin microcapsules in aqueous pH 4 dan 7. Maltodextrin Dextrose Equivalent 10 was chosen as encapsulant with core to coating ratio 1 : 10 and 1 : 20. Stability of limonin microcapsule in aqueous pH 4 and 7 was investigated for 14 days. Microencapsulation was conducted using freeze dryer. Microcapsules surface was analyze using Scanning Electron Microscope and the result show limonin microcapsule with core to coating ration 1 : 20 had better appereance and uniformity in size. Limonin content was measured using spertrofotometry method. Result showed limonin microencapsulation with core to coating ratio 1 : 10 had efficiency of 81,3% while ratio 1 : 20 had efficiency of 83,5%. Maltodextrin was unstable at aqueous pH 4 and limonin in microcapsule with core to coating ratio 1 : 10 start released at day 6. While limonin microcapsule with core to coating ratio 1 : 20 start to release on day 4 at aqueous pH 4 and on aqueous pH 7, microcapsule start to release on day 10.

1. Introduction
Consumers demand on food that provide both nutritional value and health benefit continue to grow. Research on plant bioactive compound continue to develop to identified the benefit of bioactive compound that in the future can be used as food as well as drugs.[1] There are 39 limonoid aglycone and 21 limonoid glucoside have been isolated from citrus. Limonin is the main flavonoid in citrus plant and the main caused of delayed bitterness in citrus fruit.[2] Limonin is highly oxygenated triterpenoid compound that study in mice showed limonin can inhibit cancer cells.[3] Limonin has antioxidant capacity that 2,9 -8,3 higher than vitamin C.

Bioactive compound with potential health benefit have several limitations such as unstable, heat sensitif and oxigen sensitif. These factors cause shelf life and bioavalabilty of bioactive compounds in food are limited. [4] Technology needs to be applied to overcome these problem, many researchers proposed microencapsulation. Scientific review conducted by Dias, Ferreira, & Barreiro, showed application on microencapsulation on bioactive compound had stabilize and protect bioactive into core therefore added the shelf life of bioactive compound itself.[5] Microencapsulation of bioactive compound from red grape can reduce bitterness as well as stabilize polyphenols content. [6] Microencapsulation process commonly using spray dryer or freeze dryer.[7] They combine these two
methods and the use of spray-freeze drying method produce DHA powder with low peroxide value and spherically shape with fine surface's pores.[8] Microencapsulation using freeze dryer result in product with better organoleptic characteristic and bioavailability since changes caused by heating are minimized.[9] Lycopene microencapsulation offered better stability against heat and oxygen than its free form. microcapsules also able to release homogenous pigment in food system.[10] Microencapsulation also helps alter desirable sensory attributes of bioactive compound. As in limonin, microencapsulation also helps to reduce bitterness of limonin. [11] Limonin microencapsulated can cause problem for biocative compound that sensitive to heat.[12] Therefore, in this research microencapsulation process was using freeze dryer to prevent damage of bioactive compound cause by heat also to produce uniform microcapsules.

There are several materials can be used as coating in microencapsulation from variety of polymer and non polymer.[13] Maltodextrin with Dextose Equivalent between 10 to 20 can be used as coating material for encapsulation and give satisfactory result.[14] Freeze dried microencapsulation process of argentine red wine using maltodextrin DE 10 as coating material resulted in no loss of poliphenol content of wine powder after 15 days storage at 38oC. [15] Microencapsulation of sour cherries using maltodextrin and gum arabic with core to coating ratio 1 : 20 resulted in higher encapsulation efficiencies and smaller particle size than ration 1 : 10. [16] Microencapsulation of ascorbic acid using maltodextrin showed only 7% of ascorbic acid reduction in sample after storage for 60 days at 28oC temperature. maltodextrin showed higer protection of bioactive compound compare with gum arabic, gum Arabic–modified starch (GA–MS), modified starch–chitosan (MS–CH) and modified starch–maltodextrin–chitosan (MS–MD–CH) (Chramioti et al., 2015). Limonin microencapsulation using maltodextrin DE 10 is expected to reduce limonin bitterness and add limonin shelflife. [17]

2. Method

2.1. Material and reagents
Orange seeds was obtained from local market, reagents for limonin extraction were n-hexane, acetone, dichloromethane, isopropanol. Reagent for limonin extraction were technical grade and obtained from Brataco chemicals, Indonesia. Reagent for limonin analysis was ethanol, 4-dimethylamino benzaldehyde (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 encapsapsulation was maltodextrin with DE 10 with purification 99% and obtained from Sigma aldrich, USA. Aqueous pH 4 and 7 were made by adding citric acid monohydrate and disodium hydrogen phosphate to aquadest. All chemicals used for making aqueous pH 4 and 7 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), centrífugator (Nesco, China), rotary vacuum evaporator (Ika, Germany), oven (Etuvès, France), analytical balance (Mettler Toledo, USA), freeze dryer (Eyela, Japan), and glasses apparatus (Isolab, Germany).

2.2. Limonin extraction
Orange seeds was dried in oven 50oC for 12 hours to reduce the water content then grounded to increase surface area. Limonin extraction method proposed by Cakrawati (2010) using several stages of soxhlet extraction using n-hexane and acetone respectively. Precipitation method was applied to get limonin crystal, it was using n-hexane then purified by addition of dicloromethane and isopropanol. Limonin extract was analyzed to determine total limonin content using method proposed by abbassi (2005) based on reaction of furan wing with burham reagent in acid condition and its absorbance was measured using spectrophotometer at wavelength of 503 nm.

2.3. Production of microcapsules
Encapsulant was made by dissolved 10 grams of maltodextrin DE 10 in 100 ml aquadest and was continuously mixed using hot plate magnetic stirer. Limonin then added by ratio 1 : 10 and 1 : 20 respectively. Limonin microencapsulation was conducted using freeze dryer for 24 hours with pressure <0.01 mmHg (<133 Pa) and temperature (-40°C-20°C). Microcapsule was weigh and stored in dessicator.

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

\[ EE(\%) = \frac{E_{LC}}{T_{LC}} = \frac{T_{LC} - S_{LC}}{T_{LC}} \times 100 \]

2.5. Analysis of Microcapsule surface using SEM
The microcapsule surface were analyze by scanning electron microscope (SEM) using JSM-6510 (JEOL, Japan). The samples were paled on metallic adhesive tape. SEM images were taken at 3000 x magnification and 10.000x magnification. The observations were made at voltage at 10 kV

2.6. 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.

2.7. Limonin stability on aqueous pH 4 and pH 7
Aqueous pH 4 was made by dissolving citric acid monohydrate into aquadest until pH value reached 4. While aqueous pH 7 was made by dissolving disodium hydrogen phosphate into aquadest until pH value reached 7. Limonin microcapsule was added at concentration of 2% and stored in bottle made of glass at room temperature. limonin concentration on aqueous was measured every 2 days for 14 days. The measurement of limonin concentration on aqueous based on method proposed by Setyadjit (2005).

3. Results and discussion

3.1. Scanning Electron Microscopy
Scanning electron images of limonin microcapsule prepare from maltodextrin with core to coating ratio 1 : 10 showed the shape that not uniform as seen in figure 1. Morphological variations such as structure and size, happens during dying process due to thermal expansion inside drying particles, depression and external fracture. Maltodextrin structure analyze in SEM showed formation of amorph glass-like formation but shape tend to be uniform. Core concentration did not interfere on particle morphology.

Scanning electron images of limonin microcapsule with core to coating ratio 1 : 20 showed microcapsules with uniform formation with particle size of 1 µm as seen in Figure 2. While limonin crystal size is 0.84µ with crystal size 0.28 x 0.22 x 0.15 µ. Limonin microcapsules morphology considered as matrix type since it has bioactive compound integrated within the matrix of coating material. It is depends a lot on the coating material selected and microencapsulation methods that used. The use of maltodextrin with low molecular weight can act as plastisizer that prevent shrinkage of the microcapsule's surface and promote spherical and smooth -surface microcapsules.
**Figure 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 x magnitude

**Figure 2.** SEM Images of limonin microcapsules (c) with core to coating material ratio of 1:20 at 3000 x magnitude; (d) with core to coating material ratio of 1:20 at 10,000 x magnitude
3.2. Encapsulation efficiency

Total limonin content in precipitate and microcapsule were determine by the method describe in Material and Method section. Total limonin content of precipitate was 121,343 mg and total limonin content of microcapsule with core to coating ratio of 1 : 10 dan 1 : 20 were determine as 22,668 mg and 20,018 mg respectively.

Determination of limonin on the microcapsules surface was needed to calculated microencapsulation efficiency. The higher limonin content on microcapsules surface means lower encapsulation efficiency because it could be assumed that limonin was not integrated in the matrix. There was no significat difference (p ≤ 0,05) between core to 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 81,32% and 83,5% respectively. Microcapsules with core to coating ratio of 1 : 10 has efficiency of 69,38 to 77,83 and microcapsules with core to coating ratio of 1 : 20 has efficiency of 78,8 to 92,26%. Microcapsules with core to coating ratio of 1 : 20 was expected to have higher efficiency since better matrix encapsulation was formed.

3.3. Solvent Residue Analysis

Since limonin extraction was using several organic solvents, it important to analyze residue of the solvents if limonin microcapsule will be added to food system. Solvent residue analysis was conducted using Gas Chromatography- Mass Spectrofotometry by dissolving limonin microcapsules into chloroform. The use of GC MS for analyzing solvent residue also reported by and the result showed choosing ideal solvent for GC analysis was important to analyze residue solvent. Therefore author choose of chloroform was based on its miscibility with other solvents which were n-hexane, acetone, dicloromethane and isopropanol.

The result was no solvent residue was detected. The GC-MC Chromatogram can be seen in Figure 2.

![Chromatogram of Solvent Residue Analysis](image)

**Figure 3.** Chromatogram of Solvent Residue Analysis
3.4. Stability of Limonin Microcapsule with core to coating ratio of 1: 10

There are three different mechanisms of biocative released from microcapsules microcapsule rupture wall, dissolution of the wall and diffusion through the wall. Stability of limonin microcapsule with core to coating ratio of 1 : 10 was measured every 2 days for 14 days and the result can be observed in Figure 4.

Microcapsules prepared from maltodextrin with low molecular weight provide greater protection on polyphenols. In normal condition (room temperature and humidity 50-60%, no light access), microcapsules can be stored for few weeks. Limonin was most stable in pH 5-7 buffered solution showed that zwitter ionin form in the least susceptible. Limonin with strong acids cause an attack to the furan. According to release mechanism of microcapsule, limonin released from microcapsules caused by mechanical-microcapsules rupture. In aqueus pH 7, less limonin was released due to stability of maltodextrin in neutral pH. Maltodextrin tend to form gel in the neutral pH condition, Limonin in the aqueous probably release from surface of microcapsule. Limonin has furan ring that easy to oxidized and damaged. Limonin measurement was based on complex compound as a result of reaction between furan ring and 4-dimethylaminobenzaldehyde. Oxidation of furan ring make it can not reacted with 4-dimethylaminobenzaldehyde therefore limonin was undetected.

In aqueous pH 4, maltodextrin as coating material started to rupture at day 4, releasing limonin into the solution. Since limonin stable at acidic pH value, it remain in the solution and the concentration was added by another limonin released from microcapsules and reached its peak on day 10. Limonin has storage time of 6 days before it start to degrade so on day 14, limonin concentration on the aqueous start to decrease. Concentration of limonin microcapsule with core to coating ratio 1 : 10 decrease 58% under acidic pH condition and 25% under netral pH. Limonin glucoside (LG) is resistant to hydrolisis to form bitter limonin. Storage of beverage contain limonin glucoside showed limonin decreased 42% and 37% under storage condition of room temperature and glass-door refrigerator respectively.

![Figure 4. Stability of Limonin microcapsule core to coating ratio 1: 10](image-url)
3.5. Stability of Limonin Microencapsule with core to coating ratio of 1: 20

Stability of limonin microcapsule with core to coating ratio of 1: 20 was measured every 2 days for 14 days and the result can be observed in Figure 5. In the microencapsulation process, some of limonin was at the surface of microcapsules, that, on the second day of storage, it start to leakage and caused addition of limonin concentration in solution. Limonin microcapsules with core to coating ratio 1: 20 reach its highest concentration on day 4.

Maltodextrin with DE 1 to 20 had lower solubility level and tend to form haze in aqueous solution pH 4. Therefore it start to dissolve in the aqueous and releasing limonin. Limonin, on the other hand, was stable at acidic pH so it remain in the solution but its concentration decrease and in day 8 no limonin was detected on solution. limonin contain furan ring what easy to oxidize therefore unable to be measured by spectrophotometer since burham reagent reaction based on reaction between 4-dimethylaminobenzaldehyd with furan ring developed complex compound with red colour. Reduction of encapsulation rate was not caused by leakage but partial deasetylation of flavonoid compound.

![Figure 5. Stability of Limonin microcapsule core to coating ratio 1: 20](image)

4. Conclusions

The result of the study reveal different limonin core to coating ratio cause different encapsulation efficiency. Encapsulation efficiency of limonin microcapsules with core to coating ratio 1: 10 and 1: 20 were 81.32% and 83.5% respectively. Morphology of microcapsules surface showed spherical form of capsules tend to uniform. Limonin was stable at acidic pH while maltodextrin was unstable, in the contrary, maltodextrin was stable at neutral pH but limonin degrade fast and this cause limonin microcapsules has different time release pattern in aqueous pH 4 and 7.

Acknowledgements

Microcapsules analysis was conducted at Lab SEM FMIPA ITB. The work is funded by Direktorat Jendral Perguruan Tinggi Ministry of Research, Technology and Higher Education Budget Year 2015.
and 2016 by virtue of Rector’s Decree No. 058/SP2H/PL/DIT.LITABMAS/II/2015 date 14 November 2014 DIPA Revisiion 01 tanggal 03 Maret 2015

References

[1] Siro I, Kapolina E, Kápolna B and Lugasi A 2008 Functional food. Product development, marketing and consumer acceptance—A reviews Appetite 51 (3) 456-467
[2] Hasegawa S and Miyake M 1996 Biochemistry and biological functions of citrus limonoids Food Reviews International 12 (4) 413-435
[3] Kuttan G, Pratheeshkumar P, Manu K A and Kuttan R 2011 Inhibition of tumor progression by naturally occurring terpenoids Pharmaceutical biology 49 (10) 995-1007
[4] Ubbink J and Krüger J 2006 Physical approaches for the delivery of active ingredients in foods Trends in Food Science & Technology 17 (5) 244-254
[5] Dias M. I, Ferreira I C and Barreiro M F 2015 Microencapsulation of bioactives for food applications Food & function 6 (4) 1035-1052
[6] Giovinazzo G and Grieco F 2015 Functional properties of grape and wine polyphenols Plant foods for human nutrition 70 (4) 454-462
[7] Gharsallaoui A, Roudaut G, Chambin O, Voilley A and Saurel R 2007 Applications of spray-drying in microencapsulation of food ingredients: An overview Food Research International 40 (9) 1107-1121
[8] Baldelli A, Boraey M A, Nobes, D S and Vehring R 2015 Analysis of the Particle Formation Process of Structured Microparticles Molecular pharmaceutics 12 (8) 2562-2573
[9] Wang L and Bohn T 2012 Health-promoting food ingredients and functional food processing Nutrition, well-being and health. Rijeka, Croatia: InTech 201-224
[10] Rocha G A, Fávaro-Trindade C S and Grosso C R F 2012 Microencapsulation of lycopene by spray drying: characterization, stability and application of microcapsules Food and Bioproducts Processing 90 (1) 37-42
[11] Sun-Waterhouse D and Wadhwa S S 2013 Industry-relevant approaches for minimising the bitterness of bioactive compounds in functional foods: a review Food and Bioprocess Technology 6 (3) 607-627
[12] Murugesan R and Orsat V 2012 Spray drying for the production of nutraceutical ingredients—a review Food and Bioprocess Technology 5 (1) 3-14
[13] Kiliaris P and Papaspyrides C D 2010 Polymer/layered silicate (clay) nanocomposites: an overview of flame retardancy Progress in Polymer Science 35 (7) 902-958
[14] Madene A, Jacquot M, Scher J and Desobry S 2006 Flavour encapsulation and controlled release—a review International journal of food science & technology 41 (1) 1-21
[15] Sanchez V, Baeza R and Chirife J 2015 Comparison of monomeric anthocyanins and colour stability of fresh, concentrate and freeze-dried encapsulated cherry juice stored at 38° C Journal of Berry Research 5 (4) 243-251
[16] Lacerda E C Q, de Araújo Calado V M, Monteiro M, Finotelli P V, Torres A G and Perrone D 2016 Starch, inulin and maltodextrin as encapsulating agents affect the quality and stability of jussara pulp microparticles Carbohydrate Polymers 151 500-510
[17] Shahidi F and Han X Q 1993 Encapsulation of food ingredients Critical Reviews in Food Science & Nutrition 33 (6) 501-547