

Encapsulation of Soursop (*Annona muricata* Linn.) Leaf Tea Extract using Natural Mucilage

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Abstract

The bioactively rich soursop leaf extract can be encapsulated inside several natural coating materials. The extract of soursop leaf has been found to contain many bioactive compounds such as various phenolic compounds that are beneficial for anticancer, antidiabetic and many other health benefits. However due to the sensitive nature of the bioactive molecules, encapsulation procedure was applied to prolong the activity of the biomolecules. Capsule materials used in this experiment were obtained from natural extract of Okra mucilage, Aloe vera mucilage and Maltodextrin-WPI mixture to protect the unstable bioactive compounds. Homogenation time using ultrasonication and Core to Coating ratio was differed to observe the optimum encapsulation process. The encapsulation efficiency, release factor ability, size distribution and its correlation with bioactive stability are observed using Total Phenolic methods and Antioxidant Activity before and after encapsulation. Aloe vera gave best encapsulation efficiency (88-91%) while microcapsule made with Okra gave the best antioxidant activity (DPPH IC₅₀ 633-710ppm), and maltodextrin-WPI gave best powder recovery (yield 70-73%).

Keywords: aloe mucilage, antioxidant activity, encapsulation, okra mucilage, soursop tea extract

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INTRODUCTION

The extract of soursop leaf was found to impart an anti-inflammatory activities, antioxidant activities and antidiabetic activities, which suggests many potential therapeutic purposes (Adewole & Ojewole, 2009; Adewole & Caxton-Martins, 2006; Bridgemohan, *et al*, 2015). The leaves of soursop contain flavonoids, tannins, saponins, phytosterols, calcium oxalate, carbohydrates, vitamin A, B and C,

phosphor and alkaloids. Soursop leaves which were processed into unfermented tea has been found to contain many bioactive compounds such as antioxidant, anticancer, antidiabetics, anti-inflammatory, and prevent kidney damage from soursop leaves ethanol extracts (Badrie & Schauss, 2009; Sousa, *et al*, 2010). Microencapsulation is believed to be effective in protecting bioactive components, preserving their stability during

processing and storage and preventing undesirable chemical reactions with other food matrix (Fang & Bhandari, 2012; Wandrey *et al*, 2012).

Okra mucilage is an acidic polysaccharide that possess high amount of rhamnose (22%), galactose (25%), galacturonic acid (27%), amino acids (11%) and low amount of arabinose, mannose and xylose (Sinha *et al*, 2014; Ameena, *et al*, 2010). Aloe vera mucilage is also been widely used as a pharmaceutical excipient, containing several natural coating materials such as; acemannan, pectin, arabinorhamnogalactan, galactan, galactogalacturan, lucogalactomannan, galactoglucoarabonimannan, and glucuronic acid (Nair 2013; Wandrey *et al*, 2012). Both natural mucilage was already proven to be a potential pharmaceutical excipient and are superior in their ability to sustain drug release mechanism (Bhaskar, *et al*, 2013). However, the protective nature of those capsule material againsts bioactive compound still need to be observed especially againsts several encapsulation conditions. In this research, the coating agent for the encapsulation will be prepared from two natural sources (mucilage of aloe vera and okra) with maltodextrin-whey protein isolate mixture as a comparison. The ratio of core material and coating material and homogenization time will be observed as factors to obtained optimum encapsulation condition.

RESEARCH METHODS

Materials and Equipment

The materials that are used in this research are fresh Soursop leaves (*Annona muricata* L.), fresh Aloe vera, and fresh Okra (*Abelmoscus esculentus*) fruits, Maltodextrin, Whey Protein Isolate. Reagents that are used in this research are Ethanol, DPPH solution, Folin-ciocalteu reagent, Sodium Carbonate, Gallic acid. The equipment used for making the extract and for the encapsulation process are incubator shaker, analytical balance, oven, cabinet dryer, glassware, roller, blender, desiccator, spray dryer, magnetic stirrer, water bath, heater, ultrasonicator, buchi mini spray dryer, and rotary evaporator. Meanwhile, the equipment used in the analysis are uv-visible wavelength spectrophotometer, microscope.

Research Methodology

The research procedures will be divided into two stages, first will be the extract preparation and the encapsulation of the extract. The first step concentrates on the preparation of aqueous extract of unfermented soursop leaf (core material) and *Aloe vera* and Okra mucilage (coating material). The second step will be concentrated on the encapsulation of the core material with the coating material, by assessing the effect of core to coating ratio and the ultrasonication time of the mixture. The soursop leaves green tea extract will be accessed for its total phenolic content, and antioxidant activity, while the encapsulated product will be accessed for its particle

distribution size, encapsulation efficiency, powder recovery, and its stability.

Preparation of Soursop Leaves Green Tea Extracts

In this section, the fresh soursop leaves will be treated to become green tea by undergo several steps. The preparation of the soursop leaves green tea extracts are adopted from Mulyawan (2007). Fresh soursop leaves are sorted and cleaned with running water, heat at 100°C for 6 minutes to fixate the enzyme, rolled at room temperature and then do the final drying using cabinet dryer at 70°C until reach 3–5% moisture content. After the soursop leaves green tea are obtained, the extraction process to extracts the phenolic component of the green tea will be done. The extraction process of the soursop leaves green tea is adopted from Adewole and Martins (2006). The obtained soursop leaves green tea extracts will be further analyzed in term of antioxidant capacity and total phenolic content (TPC). Extraction by maceration with 96% ethanol (1:10) for 48 hours in room temperature (25°C) and concentrated using rotary evaporator.

Extraction of *Aloe vera* Mucilage

The procedure of which the *Aloe vera* mucilage is extracted is adopted from the method described by Nair, 2013. An amount of fresh Aloe vera were initially weighed and then washed in running water to remove dirt and the yellowish substance. Subsequently, the flesh is sliced thinly to increase surface area. The slices are then heated in hot distilled water (70°C) with a ratio of 5:1 (water: aloe vera) for 4 hours. After heating, the mixture is settled for 2 hours to release the mucilage into the water. The mixture is then filtered using filter cloth to remove any physical contaminants (Shah & Seth, 2011).

Extraction of Okra Mucilage

Fresh okra fruits were bought in local market in West Jakarta in the morning of the day of extraction. An amount of fresh okra fruits (pods) were initially weighed and then washed in running water to remove dirt. Subsequently, the pods are sliced thinly to increase surface area. The slices are then soaked in warm (70°C) distilled water with a ratio of 5:1 (water: okra) for 4 hours. To increase the yield, the mixture is heated in the microwave oven with 360 watts for 3 minutes (Shah & Seth, 2011; Lim, 2014). After being heated, the mixture is let settle for 2 hours to release the mucilage into the water. The mixture is then filtered using filter cloth to remove any physical contaminants. The resulting okra mucilage is then ready for the subsequent processes.

Encapsulation Process

The soursop leaves green tea extract (from now on will be referred as core) is weighted based on the dry weight and dissolved in 100 mL distilled water (0.1 gr/mL). This is done three times, in three different

beaker glasses. The mucilage extract (from now on will be referred as coating) is weighted based on its dry weight to three different weights: 10, 20, and 30 grams, in order to reach the core to coating ratio of 1:10, 1:20, and 1:30 (Bhaskar, *et al.*, 2013). The coating is then dissolved in 200 mL distilled water each and mixed with the dissolved core. All three of the solution is then added with distilled water to make up into 1 L of solution. The solution obtained is mixed with magnetic stirrer for approximately 30 minutes, and it is then treated with ultrasonication at 160 W power, 20 kHz frequency and with 50 % pulse for three different time periods, which are 15, 20, and 25 minutes. During sonication, samples were placed in water bath with cold water at 4 °C to prevent overheating. After all is done, the solution is then spray dried with 130 °C inlet temperature and 2.3 L/h flow rate until all the solution is end up becoming fine particles (Gharsalloui, *et al.*, 2007). The fine particles are then further analyzed for particle size, encapsulation efficiency, powder recovery, and also release factors.

Antioxidant Activity by DPPH Method

In this research, the antioxidant activity of both soursop leaves green tea extracts and the encapsulated form of the soursop leaves green tea extracts are measured by DPPH method (Mulyawan, 2007). In this method, one mL of several concentration of the core extract (10, 100, 1000 and 10000 ppm) is diluted in ethanol and is mixed with 4 mL of 0.025 g/L DPPH. Then the solution is left under dark condition for 30 minutes at room temperature. After 30 minutes, the absorbance of the solution is then measured using spectrophotometer at 517 nm. Ethanol are used as the control. The DPPH radical scavenging ability is calculated with the following formula

$$\%RSA = \left(\frac{Ac - As}{Ac} \right) \times 100\% \quad (1)$$

%RSA stands for radical scavenging ability (%), Ac stands for absorbance of the control, As means absorbance of the sample.

IC₅₀ value is the concentration of the sample that is required to inhibit 50% of the DPPH. This method is mainly used to determine the antioxidant capacity. This value can be obtained by creating a linear regression equation ($y = ax + b$) between sample concentration and %RSA with several concentration points and taking the value of $y = 50$ to determine the x value. The x value is the value of IC₅₀ of the sample.

Total Phenolic Content (TPC)

The total phenolic content of both soursop leaves green tea extracts and the encapsulated form of the soursop leaves green tea extracts are measured by the modified Folin-Ciocalteu method. In this method,

encapsulated powder is dissolved in suitable aliquot, and is made up to 0.5 mL with distilled water. Then 0/25 mL of 1 N Folin-Ciocalteu reagent along with 1.25 mL of sodium carbonate solution are added into the solution. The solution is vortexed and incubated for 2 hours. The sample is check for absorbance at 765 nm. The procedure is repeated, but using the solution used for the preparation of encapsulation, rather than the dissolved encapsulated powder. Gallic acid is used as the standard, and the obtained amount of total phenolic content is expressed as mg/L of gallic acid equivalent in 100 grams sample (Ameena, 2010; Adri & Hersoelityorini, 2013).

Encapsulation Efficiency

The encapsulation efficiency (EE) is the ratio of encapsulated phenolic content to total phenolic content (Jafari, *et al.*, 2008). Encapsulation efficiency is calculated as the amount of total phenolic content in encapsulated powder, divided by the total phenolic content of the solution used for the preparation of encapsulation. The equation is as follow:

$$EE = \frac{T_p}{T_s} \times 100 \quad (2)$$

EE means encapsulation efficiency (%), T_p means total phenolic content in the encapsulation powder and T_s means total phenolic content in the solution used for the preparation of the encapsulation.

Release studies

The release studies of polyphenols component from the encapsulated soursop leaves green tea extracts are performed by suspending 5g of the encapsulated soursop leaves green tea are suspended in 12.5 mL distilled water (Jafari, *et al.*, 2008). Then the sample was agitated using magnetic stirrer. At defined time intervals (0,5,10,20,30,40,50,60), an then a suitable amount of aliquot was taken for analysis of total phenolic content with the same method as described in the section before.

Moisture Content (MC) and Powder Recovery

5 g of samples is put into evaporating dish in which the constant weight has already been determined previously. Then, the sample is dried in the oven with temperature of 105°C for 6 hours. Moisture content is expressed as % wet basis and expressed by the following formula:

$$MC = \frac{A-B}{A} \times 100\% \quad (3)$$

MC stands for moisture content (%); A stand for the weight of initial sample before drying (g); B is the weight of final sample after drying (g).

The dry weight of the powder is expressed by the following formula (Martinez, *et al*, 2010):

$$\text{Dry weight} = \text{Total weight} \times (100\% - \text{Moisture Content} (\%)) \quad (4)$$

The powder recovery of the spray drying process is evaluated by determination of the ration between the total recovered product mass and the mass of total dissolved solid (TDS) that are initially fed into the system. The determination of powder recovery is using following equation:

$$PR = \frac{xdb (W_2 - W_1)}{T_s} \times 100\% \quad (5)$$

PR = Powder Recovery (%), Xdb = moisture content in dry basis (db), Ts = total dissolved solid (g dry matter /L), W₁ and W₂ stands for weight of the powder before and after spray dried respectively.

Particle Size

The powdered form of soursop leaves green tea extracts will be observed for its particle size by scanning electron microscopy (SEM) (Martinez, *et al*, 2010). The samples that will be sent into LIPI for further analysis is the one that has lowest particle size under the microscope observation.

Liquid Chromatography-Mass Spectrometry

For the LC-MS analysis, one gram of microcapsules was extracted with maceration using ethanol as the solvent (Champy, *et al*, 2009). The extract was then filtered and was used for the LC-MS analysis. Both core and microcapsules extracts were subjected to LC-MS analysis using Mariner Biospectrometry with 2 μ L volume injection, 0.05 mL/min flow rate, 85% ethanol eluent for 60 minutes. The column temperature was maintained at room temperature (25°C).

Experimental Design

First Stage; The experimental design in of this experiment is completely randomized factorial design with two factors A x B (3 x 2). The factors observed include: A: Type of Carrier agent (A₁, A₂ and A₃) and B: Core to coating ratio (B₁, and B₂)

Table 1. Sample combination - Encapsulation

| Carrier agent ratio | Core to coating ratio | |
|------------------------------------|-------------------------------|-------------------------------|
| | 1:10 | 1:20 |
| Aloe vera Mucilage | A ₁ B ₁ | A ₁ B ₂ |
| Okra Mucilage | A ₂ B ₂ | A ₂ B ₂ |
| Maltodextrin: Whey Protein Isolate | A ₃ B ₁ | A ₃ B ₂ |

The experiment in this research is performed twice as

replication, so the amount of the sample needed will be (3 x 3) x 2 = 18 samples. The combination of the treatment used may be seen on Table 1.

RESULTS AND DISCUSSION

Soursop leaves are well known for its high phenolic components responsible for its antioxidant activities. Soursop leaves which were processed into unfermented tea has been found to contain antioxidant, anticancer, antidiabetics, anti inflammatory, and prevent kidney damage that are proven in vivo from soursop leaves ethanol extracts (Lim, 2014). It is rich with many alkaloids and acetogenin compounds. The core (soursop leaves tea extracts) and the coating (maltodextrin-whey protein isolate (WPI), Aloe mucilages and Okra mucilages) were prepared and analyzed for its moisture content, yield, total phenolic content, and antioxidant activity. The IC₅₀ value describe the amount of antioxidant compound needed to approximately reduce 50% of the free radical. All of the extract components and the encapsulated extract were then incubated with DPPH. The extract will act as a hydrogen donor, which neutralize the unstable form of DPPH shown by the loss of violet color.

The result of the core and coating material analysis can be seen in Table 1. From the data shown, it can be concluded that the material which mainly contributes to the total phenolic content and antioxidant activity for the feed solution and microcapsules comes originally from the core material, which is soursop leaves tea extract. Maltodextrine-wpi, and aloe vera mucilage show low amount even negligent amount of both phenolic compound and antioxidant activity compare to the core extract. Soursop leaves contains acetogenins, annonacin, annonacinone, solamin, corosolone oils (sesquiterpene hydrocarbons (E)-caryophyllene, δ -cadinene, α -humulene, phenylpropanoid eugenol, sesquiterpenoid caryophyllene oxide alkaloids lactone, soquinolin, and anthraquinone (Adewole & Ojewole, 2009; Badrie & Schauss, 2009; Bridgemohan, *et al*, 2015; Sousa, *et al*, 2010).

The microcapsules are subjected to the analysis of powder recovery, total phenolic content, encapsulation efficiency, antioxidant activity, and particle size analysis. The microcapsule produce using maltodextrine combined with whey protein isolate is compared with microcapsule produced from the Aloe vera and okra mucilages. Table 3 contain the physicochemical analysis of the microcapsule produce.

Based on Table 3, it is inferred that both cores to coating ratio and capsule material affects the characteristic and yield of the microcapsules significantly ($\alpha \leq 0.05$). In which all microcapsules with 1:20 core to coating ratio has higher powder recovery percentage, better encapsulation efficiency, better antioxidant activity and smaller particle size compared to microcapsules with 1:10 core to coating ratio.

Table 2. Properties of core extract and coating material

| Sample | Yield (%) | T Phenolic Content (mgGAE/g Sample) | IC ₅₀ (PPM) |
|-----------------------------|-----------|-------------------------------------|------------------------|
| Soursop Leaves Tea Extracts | 1.989 | 149.864 | 104.779 |
| Maltodextrin-WPI | - | - | >10000 |
| Aloevera mucilage | 1.346 | - | >5000 |
| Okra mucilage | 0.891 | 36.392 | 780.263 |

Table 3. Physicochemical analysis of core extract and coating material

| Micro capsule | Core to Coating Ratio | PR (%) | IC ₅₀ (ppm) | EE (%) |
|-------------------|-----------------------|--------------------|------------------------|-----------------------|
| Aloe vera | 1:10 | 56.92 ^b | 960 ^c | 91.31 ^c |
| Aloe vera | 1:20 | 48.95 ^a | 1072 ^d | 88.10345 ^c |
| Okra | 1:10 | 51 ^a | 633.86 ^b | 82.2952 ^b |
| Okra | 1:20 | 58.79 ^b | 710.87 ^b | 83.1949 ^b |
| Maltodextrine/WPI | 1:10 | 70.05 ^c | 118.15 ^a | 71.26 ^a |
| Maltodextrine/WPI | 1:20 | 73.09 ^c | 121.6 ^a | 74.33 ^a |

PR= Powder Recovery EE= Encapsulation Efficiency

Different type of capsule material is also shown to give different result protection toward the phenolic content and the antioxidant activity of the resulted capsule.

Combination of both whey protein and maltodextrine has the highest recovery yield, which show the material ability to bind or protected more phenolic compounds. Whey proteins modified the surface properties of the particles by reducing the stickiness between the particle and dryer wall which in turn will increase the powder recovery of the powder Gharsalloui, *et al.*, 2007. The combination of maltodextrin and whey protein isolate as a coating or carrier agent, give highest antioxidant capacity compared, because it has better emulsifying and oxidative stabilities compared to other capsule material (Qilong, *et al.*, 2013).

Both natural capsule material of Aloe vera and Okra showed superiority in encapsulation efficiency, meaning that both substances can bind higher percentage of the phenolic content compare to maltodextrin-WPI material. This Natural gum with higher degree of stickiness creates a denser material with heavier cross linkage of molecules; therefore, it is able to hold the phenolic content more efficiently (Bhaskar, *et al.*, 2013).

Okra mucilage is an acidic polysaccharide that possess high amount of galacturonic acid (27%), galactose (25%), rhamnose (22%), amino acids (11%) and low amount of arabinose, mannose and xylose (Lim, 2014; Sinha, *et al.*, 2014). Okra mucilage, in a mixture with mucilage of *Hibiscus rosasinensis*, was found to be useful as an alternative binder to starch (Ameena, *et al.*, 2010). *Aloe vera* mucilage consists of

the acemannan, which is a polymer of repeating mannose units, with acetyl group present irregularly. These are groups of big β -linked polysaccharides that need form a mucilaginous substance which explain the appearance of gel like substances. Other polysaccharides found in the mucilage are asarabinan, arabinorhamnagalactan, galactan, galactogalacturan, lucogalactomannan, galactoglucoarabonimannan, and glucuronic acid (Nair, 2010; Valverde, *et al.*, 2005).

Okra gum was proven to be a potential sustained drug release polymer-bends with sodium alginate in the development of controlled glibenclamide release beads (Sinha, *et al.*, 2014). Both Okra and Aloe gums are known to contain a high presence of mannose residues. Recent studies showed that the epithelial cells lining the digestive tract have mannose-specific receptors for the active uptake of mannose from the digestive tract and transporting it to the blood stream up to 90 minutes after direct consumption of the mucilage. The high affinity of these mucilage toward binding of the phenolic compound professed its ability toward a potent drug delivery (Nair, 2010).

However, the presence of high polymer carbohydrate also posed a problem in the high adhesiveness of the compound.

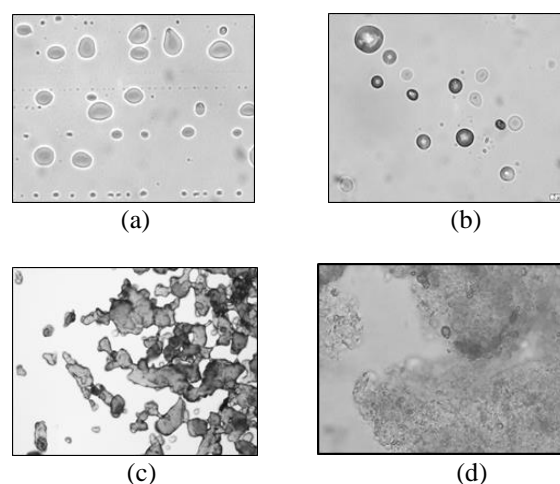


Figure 1. Microscopy image of the microencapsulation of (a) maltodextrine-WPI empty capsules (b) maltodextrine-WPI capsules with phenolic compound, (c) the mucilages of okra and (d) the mucilages of aloe vera.

From the images of the capsule from different sources of material, maltodextrine/ whey protein capsule exhibits an almost uniform capsule forms, however the aloe and the okra capsules showed numerous clustered regions. There are obvious appearances of clumping molecules with gel form observed in the microscopy image. Despite the high binding capacity of both mucilages, these clumped structures suggested the lack ability of the natural mucilage to fully enclose the extract in capsule form. Due the lack of the ability to form a protective barrier around the phenolic compound, the capsule derived from natural sources, such as aloe and okra mucilages shown to have less ability in the protecting the antioxidant activity of the extract (Bhaskar, *et al*, 2013).

Encapsulation using maltodextrine and whey protein isolate, can maintain the antioxidant activity of the sole extract, better than the microcapsule from natural mucilages. Statistically there is no difference in the radical scavenging activity of the sole extract with the maltodextrine-wpi capsule form. While both aloe and Okra mucilages capsule showed somewhat loss in the antioxidant activity of the core extract. Ratio of Extract and Capsule showed no effect toward the preservation of antioxidant activity from all sample.

Different capsules material is not just affecting the antioxidant activity but also affecting the releasing factors of the phenolic content of the capsules. All the microcapsule was mixed with buffer solution and stirred for some period. The amount of phenolic compound released per time is considered as the release factor of the microcapsule.

All the capsules exhibited different dissolution pattern which exhibited how the capsule materials clearly affecting the behavior of the capsule. The release phenolic compound from the capsule made from maltodextrine/WPI are almost instantaneous, which showed the highly susceptibility of both maltodextrine and WPI to water. Aloe mucilages albeit showed slower release factor, also release almost 100% its content by 10 minutes of observation time the capsules, release almost 100% of its content by 5 minutes of observation time.

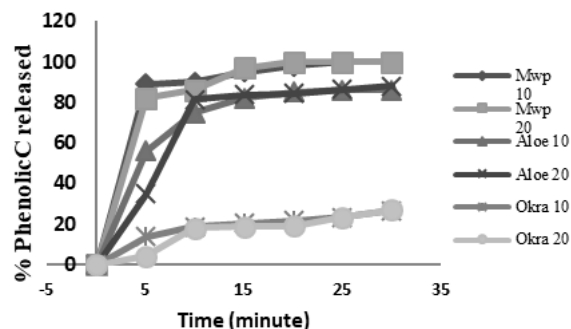


Figure 2. Release Studies of Microcapsules

Okra capsule seemed to retain the containing core material much longer than the other material. Okra mucilage originally was known to be sparingly soluble in water, although it is able to swell and forming a viscous mucilage. This high viscosity gum produces capsule with much slower release factor, which also exhibited in other research with similar feature (Zaharuddin, *et al*, 2014). Okra has been known to be able retard the release of a medicinal compound, propranolol hydrochloride, up to 24 hours after consumption. This slower release of the component might be a good characteristic of natural capsule that can be further enhance or control for better drug delivery systems.

The core extract and the microcapsules are subjected to HPLC-MS to determine the component inside. The spectrum revealed a variety of peak at various retention time, with molecular weight similar to what expected from acetogenins. Peaks at minute 4 to 11 minutes showed existence of component with molecular consistence with molecular weight from Acetogenin. Acetogenin derivatives such as murisolin, annoglaxin, and bulatacin has a various molecular weight, ranging around 500-650 g/mol (Champy, *et al*, 2009). Which means that maltodextrin-WPI microcapsules were able to retain the original core extracts component efficiently best. All three capsules showed the presence of smaller components, half the mass of the acetogenin groups, which indicated the formation of smaller compounds after heat application.

Table 4. Mass Spectrophotometry of Core Material and Microcapsules Extract

| LC-MS Peak | Core Extracts | MWP microcapsule | Aloe microcapsule | Okra microcapsule |
|--|---------------|------------------|-------------------|-------------------|
| Ratio retention Time (min) to Molecular weight (m/z) | 2.46 (154.22) | - | 2.61 (219.23) | 2.89 (219.23) |
| | 4 (614.10) | 4.05 (614.11) | - | - |
| | 4.7 (598.0) | 4.71 (598.0) | - | - |
| | 6.2 (584.03) | - | - | - |
| | - | 7.2 (372.68) | 6.9 (371.68) | 6.81 (371.67) |
| | 11.2 (548.21) | 12.12 (548.03) | - | 12.54 (548.99) |

Okra and Aloe are seen to unable completely cover the soursop leaves extract, from the microscopy image. These inability to completely cover the extract might cause the extract to have higher exposure toward heat air during spray drying process. From aloe capsule, there are no big acetogenins molecules presence, however the three capsule of okra and aloe and MWP showed the presence of similar smaller component around $T_r = 7$ minutes, which might responsible for the increase in the antioxidant activity of the capsule material.

CONCLUSION

From the research, it can be concluded that both coating material and core to coating ratio are affecting the encapsulation of soursop leaves tea extract. Microcapsules with 1:20 core to coating ratio were able to encapsulate the core material better compared to 1:10. However, the optimum coating material was yet to be determined as aloe vera gave best encapsulation efficiency while Okra gave best antioxidant activity, and maltodextrin-WPI gave best powder recovery. However in general, both aloe vera and okra mucilage were able to produce microcapsules with better antioxidant activity, encapsulation efficiency, and particle size compared to that of maltodextrin-WPI, however the powder recovery is much higher in maltodextrin-WPI compared to natural mucilage. In conclusion, aloe vera and okra mucilage are both capable in encapsulating soursop leaves tea extract, but further research needs to be done in order to increase the powder recovery of the microcapsules.

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