THE EFFECT OF SODIUM TRIPOLYPHOSPHATE LEVEL ON THE PRODUCTION OF ANTHOCYANIN MICROCAPSULES FROM PURPLE SWEET POTATOES

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Abstract - Purple sweet potatoes (Ipomoea batatas L.) is a plant rich in anthocyanin. Anthocyanin is a group of reddish pigment contained in the fluids of a cell. Synthetic food colouring substance used widely in the market poses danger to the consumers' health, and this turns anthocyanin as an alternative substance for food colouring in demand to be developed as it is a non-toxic food-grade substance.
Anthocyanin microcapsules are produced to protect the compound of anthocyanin contained in the extract of purple sweet potatoes. In the process of microcapsules production using chitosan and NaTTP as the crosslinkers, the selection of NaTTP is done because it does not dissolve in ethanol while at the same time having decent solubility in water when used for food production. To find out if the variation of adding NaTTP to the microencapsulation process affects the final result of microcapsules, the following analyses are conducted, including Fourier Transform Infra Red (FTIR), Yield of Microcapsules (YOM), and UV-Vis Spectrophotometry.
The results obtained from the aforementioned analyses include the crosslink of chitosan-TPP being on the discrepancy of wavelength for ±1155 cm-1, Yield of Microcapsules (YOM) and Total Monomer Anthocyanin (TMA) scores increasing when TPP concentration increases, with 0,3 (b/V) being the highest score of TPP concentration, while YOM score reaches 2,63 (g) and TMA score amounts to 98,73%.

Keywords - Anthocyanin, Crosslinker, Micro-encapsulation, Chtosan, Purple Sweet Potatoes

I. INTRODUCTION

The use of food colouring substance is now inseparable from the development of food industry. Colourful presentation and packaging has become one of food product specifications affecting whether or not the product is appealing to the consumers. Many industry players choose to use synthetic food colouring substances because it is more economical and produces brighter colours for their products.
Rhodamine B (used for red-coloured food products) and yellow methanil are two of synthetic food colouring substances used widely in food industry which inflict a negative side effect for the consumers' health, including abnormalities in organs and impaired digestive functions (Nasution, 2014). Therefore, the development of alternative food colouring substances which can substitute the synthetic ones is currently high on demand.
One of the alternatives to substitute synthetic food colouring substances is anthocyanin, which can be obtained from fruits and vegetables (Wang, Jung, & Zhao, 2016) and can be used to give colours for drinks, dairy products, breads, cakes, and products with preservatives (Burdock, 1997). Anthocyanin is a pigment rich in antioxidant and other natural substances that can prevent cancer, diabetes, deuro-degenerative diseases, inflammations, and post-menopausal problems (Baghci, Sen, and Bagchi, 2004).
According to Hanum (2000), purple sweet potatoes (*Ipomoea batatas L.*) is rich in anthocyanin, which can provide antioxidant and other useful substances for consumers' health. Anthocyanin contained in purple sweet potatoes is scientifically the most complete kind compared to that of red cabbages and red corns (Hambali, Mayasari, Noermansyah, 2014), which makes it a potential raw ingredient to substitute synthetic food colouring substances. One of the obstacles in using purple sweet potato-based colouring substance is its sensitivity to the changes happening in the environment, which makes the storing process a bit unstable to handle.

Micro-encapsulation is a technique in which tiny particles of solids, fluids, or gas are coated with a thin layer using polymer system to produce a capsule in order to shield active food ingredients (Dias, Ferreira, & Barreiro, 2015). This thin coating protects the ingredients inside from decomposition, reduces evaporation of active components, and prevent undesirable conditions from happening (Carlota Mendez, 2017). Natural food colouring substance contained in purple sweet potatoes has a good storage time to be commercialized for this purpose.
NaTTp is a crosslinker, and it is selected to be used in this research because of its ability not to dissolve in ethanol while having a good solubility in water, as well as its quick gel ability and its ability to act as a good absorbent (Hui Huang, 2007). According to Nussinovitch (2010) chitosan-TPP is the best crosslinker there is in the field. Adding different levels of NaTTP will show different activities. This research aims to observe the effect of different levels of NaTTP on the micro-capsulation of anthocyanin obtained purple sweet potatoes.

II. MATERIALS & METHODS

2.1 Materials

The key ingredient used in this research is purple sweet potatoes (Ipomoea batatas L.), with 3-4 days preparation time, bought from Pasar Degolan, Ngemplak, Sleman, Yogyakarta. The chemical substances used in the process include ethanol (96%), citric acid, chitosan, acetic acid with a pH of 2.6, sodium tripolyphosphate (NaTPP) (85%), potassium chloride buffer (KCl) with a pH of 1.0, hydrochloric acid (HCl) with a pH of 2.6, sodium acetate buffer with a pH of 4.5. The equipment used includes graduated cylinder, Erlenmeyer, analytical balance, blender, drop pipette, hot plate stirrer, magnetic stirrer, filter paper, aluminum foil, separation funnel, centrifuge, spectrophotometry.

2.2. Methods

2.2.1. Extraction

The extraction of anthocyanin from purple sweet potatoes is done according to the method (Reyes, et al., 2015) with several modifications. Selected purple sweet potatoes are washed, peeled, and drained. They are then sliced across with a thickness of 0,2-0,4 cm, and put into the steam-blanching process with a temperature of 100oC for 5 minutes to inactivate peroxidase enzyme. Shock cooling process is done right after. After that, they are grinded and extracted using citric acid-ethanol as the solvent, of 96% concentration (3% w/v), for 4 hours at 35℃ in a dark environment. Then the solids are separated from the supernatant using a four-layered filter paper. Filtration is followed by using vacuumed Whatman filter paper number 41. Evaporation at 50℃ is done in a vacuum condition to extract the anthocyanin for half of the volume before extraction.

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2.2.1Encapsulation Process

This process is divided into 2 stages including the preparation of polymer, and the crosslinking.

At the polymer preparation stage, anthocyanin extracts is dissolved using KCL buffer with 1:1 (v/v) composition. At the same time, 0,02 gr chitosan is dissolved in 20 ml of acetate solution with a pH of 2,6, which is then followed by Sodium Tripolyphosphate with a variation (0; 0,1; 0,2; 0,3) (b/v) or (P1, P2, P3, P4) being dissolved in 20 ml of HCl solution with a pH of 2,6.

The next stage is crosslinking, at which chitosan solution and anthocyanin extract is mixed in a separation funnel, and then titrated in STTP solution, stirred for 3 minutes, and followed by centrifugated encapsulation for 10.000 g for 10 minutes to separate the suspension and supernatant.

2.3. Chemical Analysis

Chemical analysis is done to find out the total monomer of anthocyanin (TMA) with a procedure established by Wenjie W, Jooyeoun J, and Yanyun Zhao (2016) with ph difference method (Wrolstad, et al., 2005).

1. 1 ml of supernatant from anthocyanin extract is diluted in 3 ml of KCL 9 buffer (pH=1,0) and acetate buffer (pH= 4,5)

2. The mixed solution is then vortexed and stored still at room temperature for 15 minutes.

3. Each of the solution is then tested for absorptivity using UV-1800 (shimadzu UV spectrophotometer) at both waves of 520 nm and 700 nm.

4. Diluted sample absorptivity is calculated using the following formula:

$A = (A_{520} - A_{700})_{pH\ 1,0} - (A_{520} - A_{700})_{pH\ 4,5}$.....(1)

5. 5. Total monomer of anthocyanin (TMA) is calculated using the following formula:

$$TMA\ (mg) = \frac{A \times Mw \times DF \times 10^3}{1 \times \varepsilon \times V}$$.....(2)

Description:

MW =Molar weight of cyanidin-3-glycosides (449.2)

DF =Dilution Factor

E =Molar absorptivity cyanidin-3-glycosides (26900 L/mol.cm)

L =Cell pathlength (1 cm)

6. 6. TMA recovery indicating anthocyanin encapsulation efficiency for nano-capsules is calculated using the following formula:

7.

$$\text{TMA Recovery\%} = \frac{\text{TMAanthocyanin extract} - \text{TMAsupernatant}}{\text{TMAanthocyanin extract}}$$..(3)

2.4. Yield of Microcapsule Analysis

YON analysis is a test of encapsulation mass in gram. This analysis starts with weighing empty centrifugal cylinder (m0). To separate the supernatant and the suspension, a filtration is done using the suspension in the centrifugal cylinder. Separated supernatant is then analysed while the remaining suspension in the cylinder is weighed. YON score is calculated using the following formula

$$YON = m_o - m$$ .....(4)

2.5. Fourier Transform Infrared Spectrometry(FT-IR) Analysis

To make sure there is an interaction among anthocyanin, chitosan, and TPP, a Fourier Transform Infrared Spectrometry (FT-IR) analysis on chitosan, chitosan nanoparticle without anthocyanin, and chitosan nanoparticle with anthocyanin, is needed. Fourier Transform Infrared Spectrometry (FT-IR) analysis uses FTIR Nicolet Avatar with qualitative testing method.

III. RESULT AND DISCUSSION

3.1 Effect of NaTTP Levels on Total Monomer of Anthocyanin (TMA)

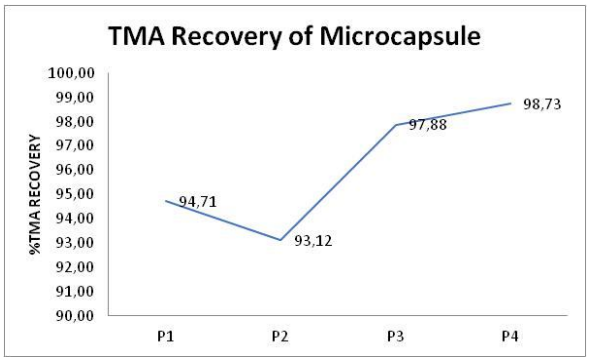


Fig.II

The amount of TMA content in the extract of purple sweet potatoes is determined by pH difference, the more NaTTP is given, the more amount of TMA there is (Dranca, Florina 2015), which is consistent with the score shown in Fig.I indicating that the higher the concentration of TPP, the higher the amount of anthocyanin absorbed. The graph illustrates that the chitosan with an NaTTP of 0,3 (b/v) is the TPP-added session that shows the highest amount of anthocyanin absorbed.

Additionally, the amount of TMA contained in the extract of purple sweet potatoes is affected by the storage time of the sample. Longer storage time of the sample affects the absorptivity score because the structure of the content in the sample is affected too, and the longer it gets, the TMA amount will increase because acidic content reacting in the sample increases too (Dranca, Florina 2015).

3.3. Effect of TPP Levels on the Structural Nature of Encapsulation Spectrum

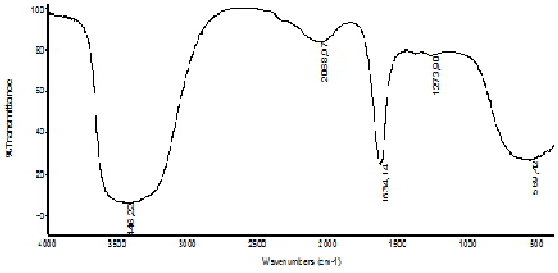


Fig.II, Sample 1 (P1)

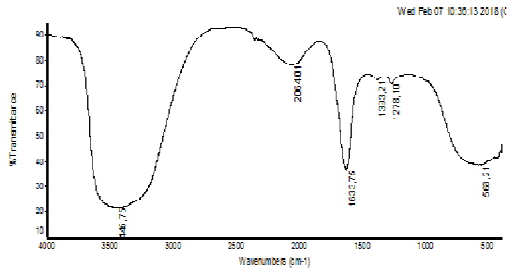


Fig.III, Sample 2 (P2)

FTIR analysis generates data that do not differ significantly. IR spectra of P1 sample indicates an uptake at a wave number of 3446,22 cm-1, showing an overlap of group spacing vibration of –OH, and N−H at a wave number of 2069,97 cm-1 of C≡ group function. At a wave number of 1634,14 cm-1, a bending vibration of N–H from the primary amide is indicated. Meanwhile, at a wave number of 569,42 cm-1, it is suspected that the anthocyanin compound found is cyanidin. Cyanidin compound experiences n-π*, n-σ* and π- π* electronic transition because it is formed at a maximum peak at a wavelength range of 400-600 cm-1. IR spectra of P2 sample obtains a wave number that has a similarity as IR spectra of P1 sample, resulting in the content contained being not too different from P1 sample.

Both samples do not indicate a crosslink of chitosan-TPP on FTIR result chart. Devika, et al., 2006 claims that the crosslink of chitosan-TPP occurs at a wave number of around 1155 cm-1. This is because the sample used in FTIR analysis is of 0 TPP so the result is not indicated on FTIR chart.

3.2. Effect of TPP Levels on Yield of Microcapsule (YOM) Analysis

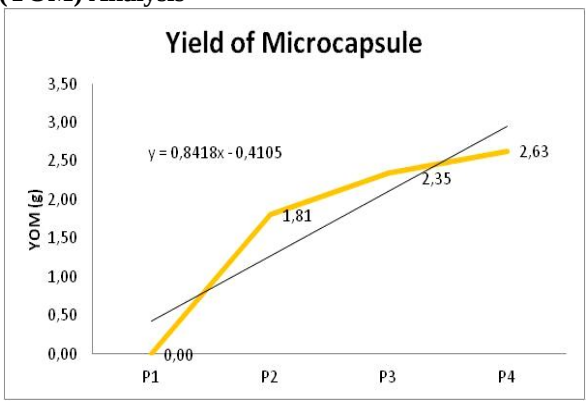


Fig. III

According to Florina Dranca (2016) YON score is affected by the amount of TPP addition to the extract. The more amount of TPP added, the higher the encapsulation yield score. In addition to that factor, YON score is also affected by the amount of the extract used, the more it is used, the higher the score is. The result score in this research is affected by the variation of the amount of TPP added, because in the encapsulation process, the amount of the extract put in the samples are of no difference.

In this research, P2 sample is the one with the lowest YON score, which is caused by the small amount of TPP added, making up the small number of binding, hence the less weight resulted (Wenjie, et al., 2016).

The highest YON score is obtained by P4 sample, in which high TPP level as the crosslinker results in high encapsulation yield score, because impaired binding process causes a significant gap, hence smaller number of chitosan binding and higher YON score.

The amount of NaTTP used as the crosslinker affects the amount of anthocyanin encapsulated. Data analysis indicates that YOM score increases as the amount of NaTTP added increases in the microencapsulation process. The illustration of the effect of NaTTP amount on the encapsulation process can be seen below.



Fig VI, Kapsul 0 NaTTP

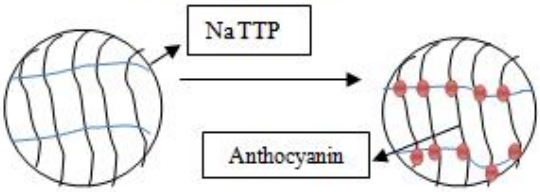


Fig VII, Kapsul 0,1 NaTTP

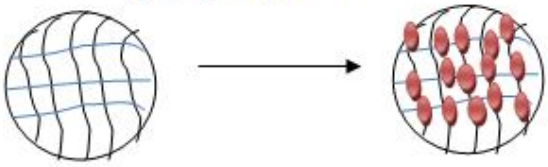


Fig VIII, Kapsul 0,2 NaTTP

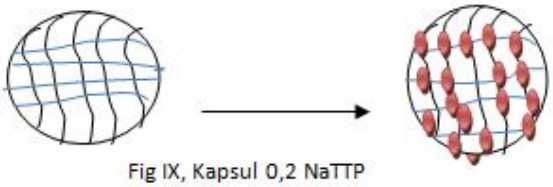


Fig IX, Kapsul 0,2 NaTTP

Above are the illustrations of encapsulation patterns with anthocyanin binding with different levels of chitosan and NaTTP. P1 is of 0 (g), because there is no crosslinker found able to bind anthocyanin contained in the extract of purple sweet potatoes, hence P1 capsule does not contain anthocyanin. Meanwhile, the amount of P2, P3 and P4 is respectively of 1,81 (g), 2,35 (g), and 2, 63 (g). Regarding the different levels of NaTTP added, the different scores of YON are shown, where P4 shows the highest and P2 shows the lowest one, in which anthocyanin binding is less than in P3 and P4.

Yield of Microcapsule analysis is conducted to find out the YOM score with different levels of NaTTP, which can be calculated using the following formula,

$y = 0{,}8418x - 0{,}4105$.....(5)

Description:

y = Yield of Microcapsule

x = Concentration of NaTTP.

CONCLUSION

Purple sweet potatoes are rich in anthocyanin, which makes them potential to be used as an alternative to synthetic food colouring substances. NaTTP as a crosslinker functions as the compound that binds anthocyanin contained in purple sweet potatoes to be used in microcapsule products.

This research studies the effect of different levels of NaTTP on the micro-capsulation process of anthocyanin, and the result concludes that a small amount of NaTTP produces microcapsules that are low in anthocyanin, and ones that have big amount of NaTTP, contains high content of anthocyanin.

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