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Physical and chemical properties of roselle extract nanocapsule with inulin, chitosan and maltodextrin as encapsulant

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Abstract

Roselle contains high phenolic compounds, mainly anthocyanins that are not stable with pH, metal ions, light exposure, temperature, oxygen, and enzymatic activity. The stability of phenolic compounds can be improved by nanoencapsulation. This research was aimed to evaluate the effect of inulin, inulin-chitosan and inulin-chitosan-maltodextrin with varying concentrations as encapsulants towards the physicochemical properties and encapsulation efficiency of nanocapsules product by spray drying. Roselle extract nanocapsules were prepared using various types and concentrations of encapsulants (inulin, inulin-chitosan and inulin-chitosan-maltodextrin). The solubility of nanocapsules ranged from 69.31 - 83.2%, while the hygroscopicity of nanocapsules was varied, approximately 17.89 - 23.79%. Nanocapsules moisture content was approximately 2.83 - 4.27%, while the total phenolic content of nanocapsules ranged from 6.74 - 13.41 mg GAE/g DW. The total anthocyanin of roselle extract nanocapsules was approximately 2.25 - 4.82 mg/g DW. The encapsulation efficiency of phenolic compounds in this study were approximately 60.31 - 77.13%. Nanocapsules with inulin-chitosan-maltodextrin (2.4%-2.4%-0.2%) had good properties of nanocapsules such as good solubility, high total phenolic content and total anthocyanin content. Nanocapsules with 5% inulin and inulin-chitosan-maltodextrin (2.4%-2.4%-0.2%) had particle size of 641.4 and 411.1 nm respectively. The nanocapsules had a spherical shape, smooth surfaces but also a few had indentations.

1. Introduction

Roselle contains high phenolic compounds, mainly anthocyanins that are known responsible for the red colour. Phenolic and anthocyanin are compounds that are not stable with pH, metal ions, light exposure, temperature, oxygen, and enzymatic activity (Bakowska *et al*., 2003). Stability is an important aspect of the use of phenolic compounds as anti-oxidants and colorants in food. The stability of phenolic compounds can be improved by using spray drying technology for nanoencapsulation (Ersus and Yurdagel, 2007).

Nanoencapsulation is one innovation in technology by means of encapsulating the active compound in an encapsulant at a very small size on the nanometer scale (0-1000 nm) (Carvajal-Zarrabal *et al*., 2009). Carbohydrates are widely used at nanoencapsulation, but research on inulin as encapsulant is still limited. The morphology of microcapsule with inulin as encapsulant has a smooth surface without cracks (Saenz *et al*., 2013). Sun-Waterhouse *et al*. (2013) also reported that microencapsulation with inulin has high encapsulation efficiency. Unfortunately, inulin's particle sizes are large, adhesive, and hygroscopic which limit its use as an encapsulant.

To produce particle size in nanoscale at roselle's extract encapsulation, the presence of inulin can be replaced in part by chitosan. In some studies, forming nanoparticles can be generated through an electrostatic crosslink between the amino group of the positively charged chitosan with negatively charged sodium tripolyphosphate (STPP) (Bahreini *et al*., 2014). Chitosan also has hygroscopic properties like inulin. To minimize the sticky and hygroscopic properties of nanocapsules, the use of inulin can be replaced in part by maltodextrin. Cai and Croke (2000) and Saénz *et al*. (2009), state that maltodextrin as an encapsulant has been used widely in the food industry because it has low hygroscopicity and high solubility. This research was aimed to evaluate the effect of inulin and inulin-chitosan

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and inulin-chitosan-maltodextrin with varying concentrations as encapsulants towards the physicochemical properties and encapsulation efficiency of nanocapsules product by spray drying.

2. Materials and methods

2.1 Materials

Dry roselle (*Hibiscus sabdariffa*) petals were obtained from a local farmer in Tawangmangu, Karanganyar, Indonesia.

2.2 Roselle extraction

Dry roselle (*Hibiscus sabdariffa*) petals were obtained from a local farmer in Tawangmangu, Karanganyar, Indonesia. Dry roselle petals were powdered using a chopper. Powdered dried roselle petals were mixed with ethanol 35% and citric acid then macerated for 24 hrs at room temperature. The ratio between powdered dried roselle petals, the solvent, and citric acid were 1:10:0.2 (w/v/w). The separation was carried out in vacuum filtration to obtain the supernatant. The roselle extract was obtained by evaporating the supernatant by using a rotary evaporator at 45°C. The extract was then stored in screw-capped inert bottle until the experiment.

2.3 Nanocapsules preparation

The encapsulants were used in the spray drying process. The encapsulants were inulin with the brand Orafti with DP >10 purchased from Orafti sales office in Indonesia, chitosan with deacetylation percent of 96.33% and maltodextrin DE 10 were purchased from Sigma-Aldrich, US. Nanocapsules preparation in this study applied Saloko *et al*. (2013) method with some modification. Chitosan (0.2% w/w) and 15% roselle extract (w/w) were dissolved in water then heated until 40°C for 15 mins. Then, STPP (0.04% w/w) was mixed with chitosan solution using a magnetic stirrer for 20 mins. Afterwards, inulin (4.8%; 9.8%; 14%; 8% (w/w)) or inulin-maltodextrin (2.4%-2.4%; 4.9%-4.9%; 7.4%-7.4% (w/w)) were dissolved completely in chitosan-STPP-roselle extract solution until became dispersion. The dispersion was homogenized using a rotor-stator homogenizer (Ultraturrax T50 Basic IKA Werke, Germany) at 6.400 rpm for 5 mins. The Spray drying process was performed at a temperature inlet of 120°C and a flow rate of 5 mL/min.

For nanocapsules made from inulin alone, inulin (5%, 10%, and 15%) were mixed with roselle extract (15%) and water using a magnetic stirrer for 5 mins. Then, the solution was mixed with STPP (0.04%) using a magnetic stirrer for 20 mins. Afterwards, the solution was homogenized and spray dried using the same treatment as the other samples. The types of the encapsulant, the concentration of encapsulant and the sample codes are shown in Table 1. The nanocapsules were characterized for solubility, hygroscopicity, anthocyanins content, phenolic compounds, antioxidant activity, microstructure, and size. All analyses were performed in triplicate and the absence of light.

Table 1. The type and concentration of encapsulants used for roselle extract nanoencapsulation and the sample codes used for this study.

Type and concentration of Encapsulant	Sample
Inulin (5%)	S1
Inulin-Chitosan (4.8%-0.2%)	S2
Inulin-Chitosan-Maltodextrin (2.4%-2.4%-0.2%)	S3
Inulin (10%)	S4
Inulin-Chitosan (9.8%-0.2%)	S5
Inulin-Chitosan-Maltodextrin (4.9%-4.9%-0.2%)	S6
Inulin (15%)	S7
Inulin-Chitosan (14.8%-0.2%)	S8
Inulin-Chitosan-Maltodextrin (7.4%-7.4%-0.2%)	S9

2.4 Analysis

2.4.1 Determination of moisture content

The moisture contents of the nanocapsules were analysed using the gravimetric method.

2.4.2 Determination of solubility

Solubility was analysed according to the study by Braga *et al*. (2019) with some adaptations, where 0.5 g of the nanocapsule was placed in beakers containing 50 mL of distilled water then homogenized for thirty mins at 100 rpm at room temperature. The nanocapsule solution was then centrifuged for five mins at 3500 rpm. The supernatant was transferred to a porcelain dish of known weight and kept in the oven (Memmert) at 105°C until the mass remained constant.

2.4.3 Determination of hygroscopicity

The methodology proposed by Braga *et al*. (2019) with some modifications was employed. As much as 0.2 g of the nanocapsule was placed in porcelain capsules, then stored in a desiccator containing sodium chloride (saturated solution) for seven days at room temperature. The result was expressed in percentage (%).

2.4.4 Determination of total phenolics

The phenolic content in the nanocapsules was determined by employing the Folin-Ciocalteu method, according to the protocol described by González *et al*. (2019) with modifications. In this procedure, 0.5 g of nanocapsule was resuspended in 20 mL of distilled

water. Then, 1 mL of nanocapsule solution was mixed with 10 mL of distilled water and 500 µL of Folin-Ciocalteu reagent and was allowed to react for 3 mins. Then, 1.5 mL of sodium carbonate (20%, w/v) were added and allowed to stand for 1 hour. The reading was performed in a UV-visible spectrophotometer (Shimadzu -1800) at 765 nm, using distilled water as blank. The results were expressed in mg gallic acid equivalent per g dry weight sample (mg GAE/g DW).

2.4.5 Determination of anthocyanins

The total anthocyanin content was determined by the pH differential (Yousefi *et al*., 2015) method, where 0.1 g of nanocapsule was added with 10 mL of distilled water. Then the solution was dissolved in KCl buffer (0.025 M, pH 1.0) and CH3COONa (0.4 M, pH 4.5) at a ratio of 1:7.5. The absorbance (A) was measured in UV-visible (Shimadzu-1800) spectrophotometer at 510 and 700 nm.

2.4.6 Determination of encapsulation efficiency

Determination of encapsulation efficiency (EE) of phenolic compound (PC) using the method by González *et al*. (2019). EE of PC was calculated according to Equation 1.

$$EE\% = \frac{Total\ PC\ content - Surface\ PC\ content}{Total\ PC\ Content} \times 100$$

For the evaluation of the total PC content, 100 mg of nanocapsule was resuspended in 1 mL of deionized water. The sample was placed in a sonication bath for 30 min at 28°C to allow the rupture of the nanocapsules in the solution. 95% ethanol (10 mL) was added and the solution was left in agitation for 30 min. The sample was centrifuged at 4500 g for 10 mins and then filtered at 0.45 µm. The determination of the phenolic content was performed by Folin-Ciocalteau.

2.4.7 Particle size measurement of nanocapsules

The measurement of particle size was done by suspending the nanocapsules in distilled water. The particle sizes were measured using a laser particle size distribution analyser (Malvern Zetasizer Nanoseries Nano ZS Ver 6.20, Malvern Instruments Ltd., Malvern, UK). The size distribution was determined by the span value (Saloko *et al*., 2014). The measurements were carried out triplicate.

2.4.8 Observation of nanocapsules morphology

The morphology of the nanocapsules obtained under optimal conditions was evaluated by scanning electron microscopy (SEM). The nanocapsules were coated with gold using a Varian Vacuum Evaporator PS 10E and analysed using a LEO 1420 VP (LEO Electron Microscopy Ltd., UK), operated at 20 kV. The images were digitally obtained using software (EDS 7424, Oxford Instruments, UK).

2.4.9 Statistical analysis

The analysis data obtained were analysed statistically using the one-way ANOVA SPSS 16.0 method. If it shows significant results then proceed with a real difference test using Duncan's Multiple Range Test (DMRT) at the significance level $\alpha = 0.05$.

3. Results and discussion

3.1 Physical characterization of nanocapsules

The physical characterizations of nanocapsules are summarized in Table 2. As observed in Table 2, the solubility of nanocapsules were approximately 69.31 - 83.2%. S1 had the lowest solubility because inulin structures have a long chain. S4 and S7 showed better solubility (S1). The greater use of inulin encapsulant concentration can increase solubility. It is associated with the high total solids that affect forming crust. The hygroscopicity of nanocapsules were approximately 17.89 - 23.79% (Table 2.). The changing of inulin encapsulant with maltodextrin can cause a decrease in hygroscopicity. Maltodextrin has fewer hydrophilic compounds than inulin. According to Saenz *et al*. (2009), maltodextrin is an encapsulant that has low hygroscopicity. S2 had the highest hygroscopicity.

3.2 Chemical characterization of nanocapsules and encapsulation efficiency

The chemical characterization of nanocapsules and encapsulation efficiency were summarized in Table 2. Nanocapsules had a moisture content were approximately 2.83-4.27%. S8 and S9 had a lower moisture content compared to S2 and S3. Bhusari *et al*. (2014) report that the addition of encapsulant concentration can increase the total soluble solid and decrease the moisture content in the nanoparticle solution. The moisture content of the nanocapsules in this study was in accordance with the research by Silva-Ibrahim *et al*. (2013) and De Souza *et al*. (2015). The moisture content of jabuticaba microcapsules and bordo grape microcapsules were approximately 2.11-5.31% (Silva-Ibrahim *et al*., 2013) and 2.96-4.94% (De Souza *et al*., 2015), respectively.

The yield of inulin is higher than Arfiani (2016) which has the highest white sweet potato yield of 5.5% and 22.53% for blanching white sweet potato inulin with 2% egg albumin treatment (Yudhistira *et al*., 2020). Inulin recovery with foam mat drying method was higher than inulin by oven drying method. Extraction Inulin

Table 2. Physical and chemical characterization of roselle extract nanocapsules

Sample	Solubility (%)	Hygroscopicity (%)	Moisture content (%)	Total phenolic content (mg GAE/g DW)	Anthocyanin content (mg/g DW)	Encapsulation efficiency (%)
S1	69.31^{a}	20.99^{b}	3.06^{a}	11.76^{e}	4.48^{c}	62.39^{a}
S2	79.19^{bcd}	23.79^{c}	4.27^{c}	13.41^{f}	4.82^{d}	60.31^{a}
S3	83.2^{d}	22.03^{b}	3.88^{bc}	13.25^{f}	4.76^{d}	72.42^{c}
S4	76.4^{b}	20.3^{b}	2.91^{a}	7.86^{bc}	3.06^{b}	62.17^{a}
S5	77.71^{bc}	21.33^{b}	3.27^{ab}	8.79^{d}	2.97^{b}	67.78^{b}
S6	81.91^{cd}	17.89^{a}	3.41^{ab}	8.68^{cd}	3.03^{b}	72.50^{c}
S7	79.36^{bcd}	20.57^{b}	2.83^{a}	6.80^{a}	2.30^{a}	65.58^{b}
S8	81.91^{cd}	20.81^{b}	2.81^{a}	6.74^{a}	2.25^{a}	65.98^{b}
S9	82.56^{cd}	18.11^{a}	2.92^{a}	7.06^{ab}	2.32^{a}	77.13^{d}

Values are presented as means. Values with the different superscript within the column are significantly different at $p \leq 0.05$.

from white sweet potato reported by Yudhistira, Suswanti and Luwidharto (2020), the best yield is 7.72% from the solvent ratio of 1:2 for 12 hrs treatment. The total phenolic content of nanocapsules was approximately 6.74-13.41 mg GAE/g DW. Total anthocyanin of extract roselle nanocapsules was approximately 2.25-4.82 mg/g DW (Table 2). S1 had the lower total phenolic and total anthocyanin content compared to S2 and S6. Inulin was less strong in protecting phenolic and anthocyanin compounds. The replacement of inulin with chitosan both chitosan-maltodextrin can improve the properties of inulin in protecting phenolic and anthocyanin compounds. A study reported that inulin was less effective than maltodextrin in polyphenol encapsulation from blackcurrant (Bakowska *et al*., 2003) also from cactus pear (Saenz *et al*., 2009). According to Robert et al., (2012), each encapsulant had different optimum parameters in the spray drying process due to the different properties of each encapsulant such as solubility and viscosity which affect the formation rate of crust on the surface of the particle. This study showed that the addition of encapsulant concentrations affected the decrease in total phenolic and anthocyanin contents. Gutierrez *et al*. (2014) also found the same phenomenon. This was associated with an increase in the total soluble solids from encapsulants. EE of PC in this study were approximately 60.31-77.13%. A previous study by Robert *et al*. (2012) shows EE of PC in spray drying process using inulin of 67.5%-96.4%, while in González *et al.* (2019), EE of PC in spray drying process using sodium alginate of 57-68%.

In this study, the EE of PC was strongly influenced by the type of encapsulation used. EE of PC produced using inulin alone was lower than EE of PC using inulin-chitosan also inulin-chitosan-maltodextrin. This showed the interaction between inulin and PC was less good when compared to the interaction between Inulin-chitosan and PC also inulin-maltodextrin-chitosan and PC. In the use of chitosan-inulin or chitosan-inulin-maltodextrin for nanoencapsulation, phenolic compounds were trapped in chitosan first then inulin or inulin-maltodextrin would coat the chitosan particles and phenolic compounds of roselle extract. Whereas, in the use of inulin encapsulation without combination with other encapsulants, phenolic compounds were trapped by inulin.

The use of chitosan encapsulants resulted in better interactions with phenolic compounds due to electrostatic bonds, hydrogen bonds, and ester bonds. In acidic conditions, gallic acid compounds (one of the phenolic compounds in roselle) were not dissociated, form ester bonds when reacted with CH2OH from chitosan, whereas when gallic acid were dissociated, COO- of gallic acid would occur electrostatic reaction with the group NH^{3+} of chitosan causing electrostatic bonds (Silva-Weiss *et al*., 2013). Palma *et al*. (2014) reported that the interaction between inulin and phenolic compounds through hydrogen bonds.

EE of PC using inulin was low, this also related to the low solubility of inulin. According to Tonon *et al*. (2010), encapsulants with low solubility is caused when bioactive compounds do not diffuse properly into particles leading to phenolic compounds accumulating outside the surface layer of nanocapsules after the spray drying process. Table 2. shows that the increase of encapsulant concentration (inulin alone, inulin-chitosan, inulin-chitosan-maltodextrin) can increase encapsulation efficiency. Based on Robert *et al*. (2012), increasing the encapsulant concentration would increase the efficiency of encapsulation because the increase in total soluble solids would affect the viscosity and solubility which would affect the formation rate of crust on the particle surface. According to Gharsallaoui *et al*. (2007), the high formation of dry outer layers causes water diffusion but still can maintain bioactive compounds.

3.3 Particle size of nanocapsules

Observation of particle size distribution was carried

out on selected products. The selected product was S3. This selection was based on the best properties of nanocapsules like good solubility, high total phenolic content, total anthocyanin content and encapsulation efficiency. Then S3 was compared with nanocapsules produced from inulin alone (S1) to determine the ability of inulin to produce nano-sized nanocapsules. The particle size distribution of nanocapsules is shown in Table 3. S1 and S3 had a particle size of 641.4 nm and 411.1 nm respectively, where S3 had a smaller size than S1. In the nanoparticle solution of S3 occurred high level crosslinking between chitosan and STPP. The presence of chitosan and STPP would produce an ionic reaction due to the interaction of the positive electron of the chitosan amino group and the STPP negative electron. Azevedo *et al*. (2014), report that the presence of strong ionic reactions contributes to the reduction in particle size. The polydispersity index of S1 and S3 were 0.281 and 0.647. S3 had a heterogeneous size. Gharsallaoui *et al*. (2007), reported that high viscosity can cause particles to have heterogeneous sizes.

Table 3. Particle size of nanocapsules

Sample	Diameter (nm)	Polydispersity index
S1	641.4	0.281
S3	411.1	0.647

3.4 Nanocapsules morphology

Observation of the nanocapsules morphology was analysed using Scanning Electron Microscopy (SEM) to know the texture, shape and surface of the nanocapsules. The morphology of the nanocapsules is shown in Figure 1. S3 had a spherical shape. Some of them had smooth surfaces, but also a few had indentations. Nanocapsules had no cracks which indicated that the crust formed is resistant to heat. The shrinkage forms due to the inlet spray drying temperature used being too low. Saénz *et al*. (2009) and Tonon *et al*. (2009), state that the particles contained in indentations are obtained through the spray drying process due to shrinking particles. Nanocapsules shrinkage was due to drastic water loss followed by cooling. Cai and Croke (2000), reported that low spray drying temperatures cause the nanocapsules to shrink until indentation occurs. The use of low inlet temperatures causes the diffusion of water particles to be slower so that the particles have more time to shrink.

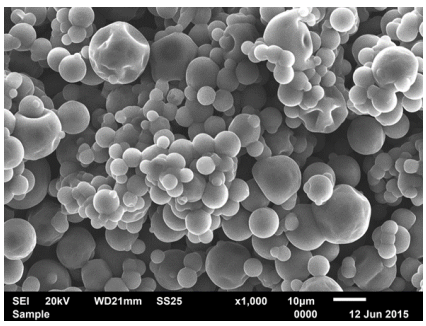

Figure 1. Nanocapsules morphology of S3

Figure 1 shows that many nanocapsules undergo agglomeration. This is due to the hygroscopic properties. Hygroscopic nanocapsules properties are associated with the use of inulin and chitosan. The presence of hydrophilic groups in inulin and chitosan encapsulants causes high hydrogen bonds between particles.

Conflict of interest

The authors declare no conflict of interest.

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