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Nano-Encapsulation of Black Rice Yeast Extract with Poloxamer Supporting Matrix

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ABSTRACT
Black rice yeast extract is rich in bioactive compounds that have the potential to be applied in the therapeutic field. Research on nano-encapsulation has a better storage period and protects against damage to bioactive components. Research on nano-encapsulation of black rice yeast extract with a poloxamer supporting matrix has been done in six stages, including (1) black rice yeast preparation and extraction of black rice yeast, (2) optimization of the sonication time on the character of black yeast rice extract (BRY-E) nano-encapsulation, (3) optimization of BRY-E and poloxamer composition supporting matrix, (4) characterization of BRY-E nano-capsule. The results showed that In terms of turbidity level, the best sonication time was 5 minutes which was marked by the smallest decrease in level turbidity during the storage period. The ratio of BRY-E and poloxamer matrix of 1:4 gave the best results, which produced particles with a size of 291 nm and zeta potential of -6.96. The best sonication time is 5 minutes.

Keywords: *Black rice yeast extract, Poloxamer, Nano-capsule.*

1. INTRODUCTION

Black rice yeast is the result of yeast fermentation grown in a black rice medium, which is known to be rich in bioactive compounds. To obtain the bioactive components, black rice yeast can be extracted using various solvents, including aquadest. The result of black rice yeast extraction using aquadest as a solvent is known as black rice yeast aquadest extract or abbreviated to BRY-E. BRY-E has identified its bioactive components, including phenolics, flavonoids, amino acids, and antioxidants [1]. To maintain a better storage period and provide protection against damage to the bioactive components of the extract, a technological is known as encapsulation can be carried out. Nano-encapsulation is a technique of coating a compound, whether solid, liquid, or gas, with a polymer to produce particles with a size of 10-1000 nm. Small nano-encapsulation has many advantages, it can protect compounds from decomposition and can control the release of active compounds [2]. An unencapsulated compound is usually unstable because it easily reacts with other compounds [3].

Nano-encapsulation has several advantages due to the presence of a polymer wall layer, so the core substance will not be affected by surroundings, prevent color and odor changes, and the stability of the core substance is maintained for a long time. In addition, it can also be mixed with other components that do not react with the core substance. Meanwhile, the disadvantage of nano-encapsulation is the coating of the core material by the polymer is not perfect or uneven and it will affect the release of the core substance from the nanocapsules [4]. Nano-encapsulation allows the active ingredient to be released periodically through the encapsulation layer, so it can increase the efficiency of active ingredient usage. Many drugs have been developed with various technologies. Currently, nanotechnology is being developed, a technology that is used to manipulate matter at atomic, molecular, and supramolecular scales.

Many polymers are used as coating materials or better known as the supporting matrix, including poloxamer. Poloxamers are a class of water-soluble non-ionic triblock ABA and BAB copolymers, where A is poly(ethylene oxide) and B is poly(propylene oxide). Poloxamers can be used in many applications that require dissolution or compounds stabilization and also have important physiological properties. The size and structure of the poloxamer assembly as well as its adsorption properties have made it useful in many applications such as drug delivery, nanoparticle synthesis, cosmetic ingredients, effective dispensing for inks/pigments, as well as as a versatile anti-biofouling coating. The use of poloxamer in pharmaceutical research has been extensively researched [5].

The method used in the manufacture of nanocapsules is the sonication method. The usage of ultrasonic waves (sonication) in the formation of nano-sized materials is very effective. The given ultrasonic waves can break up particles that previously formed bubbles that absorb the waves, and then the particles break into smaller sizes [6]. Ultrasonic radiation breaks chemical bonds in three stages. The first stage is the formation of bubbles in the sonication bath. The second stage is bubble growth which occurs through the solute vapor diffusion on the bubble, and the third stage is bubble burst which occurs when the bubble size reaches its maximum value. Ultrasonic waves do not directly interact with molecules to induce a chemical change. The interaction of ultrasonic waves with molecules occurs through an intermediary in the form of a liquid. The waves generated by electric power are transmitted by the liquid medium to the intended target [7]. The sonication time greatly affects the size of the particles that are broken down and will subsequently affect the nanosize produced. The particle size determines the stability of the nano encapsulated.

2. METHODS

2.1. Materials and Instruments

Commercial bakery yeast, black rice that is available in the market, α-amylase and glucoamylase enzymes (from China, 1000 Units, respectively), aquadest, and poloxamer 188 (from China). Some instruments: turbidimeter (TDS sensor), ultrasonic homogenizer portable (model SD-150), Malvern zeta analyzer, TEM, PSA (particle size analyzer).

2.2. Black Rice Yeast Preparation

Commercial bakery yeast, black rice that is available in the market, α-amylase and glucoamylase enzymes (from China, 1000 Units, respectively), aquadest, and poloxamer 188 (from China). Some instruments: turbidimeter (TDS sensor), ultrasonic homogenizer portable (model SD-150), Malvern zeta analyzer, TEM, PSA (particle size analyzer).

2.3. Black Rice Yeast Extract Preparation

Black rice yeast extract preparation has been done by maceration technique and using aquadest as a solvent. Maceration has been done for 24 hours and repeated 3 times. The maceration results were filtered by using a vacuum pump and evaporated by using a freeze dryer. The obtained yeast extract was used as the core material in the nano-encapsulation.

2.4. Optimization of BRY-E and Poloxamer Composition Supporting Matrix

The making of nano-encapsulated yeast was started by dissolving black rice yeast extract and matrix into 10 mL of aquadest. Yeast nano-encapsulation was made in a ratio of 1:1, 1:2, 1:3, and 1:4 (extract: poloxamer), and then dissolved in aquadest until homogeneous. The mixture was sonicated by using a probe sonicator with time variations of 5 minutes at a frequency of 30 Hz and then left in the dark for 24 hours to form a nano-capsule. The nano-encapsulation results were then centrifuged at 3000 rpm for 10 minutes and the supernatant was taken. The nano-encapsulated black rice yeast extract, hereinafter referred to as BRY-E, was stored in a closed container for characterization.

2.5. Characterization of Nano-encapsulation Yeast

The characterization that has been done in this research were 2 parameters, particle size, and zeta potential. To determine the size of BRY-E, the particle size and size distribution were measured by using a particle size analyzer (PSA). The particle size distribution was expressed in terms of the polydispersity index [8]. The zeta potential was determined to know the surface charge of yeast nano-encapsulation. The equipment used was a Malvern zeta analyzer. Determination of the zeta potential was done at a temperature of 25oC with a refractive index of 1.330; viscosity 0.8872 cP; and scattering intensity of 106.9 kcps [9].

2.6. Optimization of the sonication time on the nano-encapsule character of black yeast rice extract (BRY-E)

The manufacture of the BRY-E nano-encapsulation was carried out by dissolving 20 mg and 80 mg of poloxamer into 10 mL of distilled water. The mixture was sonicated using a probe sonicator in time variations of 0, 1, 5, 10, and 15 minutes. The mixture was incubated for 24 hours and then centrifuged at 3000 rpm for 10 minutes. The filtrate obtained was BRY-E, then tested for their turbidity level and particle size.

3. RESULTS AND DISCUSSION

BRY-E is an extract produced by the maceration process of black rice yeast using aquades as a solvent which is known to be rich in bioactive compounds. The compounds including are amino acids, phenolics, antioxidants, flavonoids, and other chemical compounds [1]. This bioactive compound has the potential to be applied in therapeutics. To protect the presence of these

bioactive compounds, nano-capsule of BRY-E was carried out using a support matrix.

3.1. Making The Process of BRY-E Nano-Capsule

In the manufacture of the BRY-E nano-capsule, a centrifugation process was carried out to separate large and nano-sized particles (<1000 nm). The particle size is determined by the encapsulation process carried out, including the centrifugation process. The comparison of the particle size resulting from the nano-encapsulation process which was preceded by the centrifugation process before sonication with the sonication process first before centrifugation was performed, is presented in Table 1.

The data in Table 1 shows that the centrifugation carried out after sonication resulted in smaller particles compared to before sonication.

Table 1. Size of BRY-E nano-capsule

Process	Nano-capsule size (nm)
Centrifuge before sonication	1074
Centrifuge after sonication	890

3.2. Optimization of BRY-E Composition and Poloxamer Supporting Matrix

The matrix used for encapsulation is poloxamer or often called pluronic, which is a non-ionic surfactant that consists of polypropylene oxide with polyethylene oxide blocks on both sides (PEO-PPO-PEO) [11]. Pluronics are tasteless, odorless, waxy, and have form as white granules that have thermosensitive gelling properties and biodegradable. Due to its strong thermal sensitivity and gel-forming ability, pluronic can be used in the development of drug deposits. The following table 2 shows the results of the PSA test based on the comparison between yeast-BH extract and poloxamers.

Table 2. PSA test results of nano-encapsulated BRY-E with poloxamer matrix

Extract Composition and Poloxamer	Measuring Temperature (^{0}C)	Particle Size (nm)	PdI
1:1	25	3,089	0.799
1:2	25	806	0.692
1:3	25	626	0.765
1:4	25	291	0.432

Measurements of the four compositions obtained, smallest size was at 1:4. This happened because a greater number of polymers can prevent agglomeration between the broken particles after stirring. Based on the data, it was known that the smallest nanocapsule size resulted in a 1:4 composition was 291 nm. Another important parameter to consider was the particle size distribution (PdI), PdI affects drug loading, drug release, and stability [12]. The particle size distribution was expressed in terms of the polydispersity index. A small polydispersity index is < 0.5 indicates that the particles have a good level of uniformity so they tend to be more stable than polydispersity. While the polydispersity category has particles that tend to form aggregates [13]. Based on the polydispersity index value obtained from the measurement of BRY-E nanocapsules, the composition 1:4 was 0.371. The smallest size BRY-E nanocapsules were zeta tested. The results are presented in Table 3.

Table 3. Zeta test results of poloxamer matrix nano-encapsulation

Sample Test Name	Duplication	Zeta Potential (mV)	Average of Zeta Potential (mV)
Yeast-BH Aquadest Extract	1	-5.06	-5.61
	2	-5.64	
	3	-6.12	
Poloxomer as Matrix	1	-11.0	-12.5
	2	-13.2	
	3	-13.3	
1:4 Ratio Nano-encapsulation	1	-5.18	-6.96
	2	-7.18	
	3	-8.52	

Determination of zeta potential was done to characterize the surface charge properties of a particle. It can be used to determine the effectiveness of surface coating or drug adsorption on nano-encapsulation [14]. The data in Table II show that the zeta value of BRY-E nano-encapsulation with poloxamer is -6.96 mV. This

value has a higher negative charge compared to the zeta value of yeast-BH extract (-5.61 mV) and a lower negative charge when compared to poloxamer (-12.5 mV). This means that the yeast-BH encapsulation process with poloxamer affects the charge value.

The Zeta potential measuring result of BRY-E nano-encapsulation at 1:4 ratio is -6.96 mV. The zeta potential value is influenced by the particle composition [15]. On the surface of the particles, the negative charge poloxamer interacts with the yeast. The large anionic charge causes the zeta potential value to be negative. In addition, the high molecular weight of poloxamer causes the zeta value to be low. The usage of encapsulants with high molecular weight, zeta values below 20 mV can provide good stabilization [12]. The usage of aquadest as a solvent also allows the particles to become negatively charged. Most particles dispersed in water become negatively charged because the adsorption prefers hydroxyl ions [16].

The factors that affect the zeta potential value are pH changes, conductivity, and concentration changes due to the addition of substances, such as ionic surfactants or polymers. The most important factor that affects the zeta value is pH, at a high pH, it will produce a low or negative zeta value, while at a low pH it will produce a positive zeta value [17].

The negative charge on the nano-encapsulation can provide lower drug entrapment but can last longer in the blood and take longer to exit into the tissue. [18]. In addition, nanoparticles that have a zeta potential close to 0 or neutral, when entering the body will more easily pass through the membrane and will not be affected by the pH in the body, so that the preparation will easily reach the target without any damage. [19].

In the zeta potential test, it is expected that the zeta value is greater than +25 mv or -25 mv because if the value is high (negative or positive), it will be electrically stable. If the zeta value is low, it will tend to agglomerate or flocculate which causes poor physical stability [20], but the zeta potential is not the main parameter in determining the stability of a nanoparticle, other factors that also can influence are size, distribution, and morphology. particles. The nano-encapsulation obtained in this research showed a value of -6.96 mV. Zeta potential is not the main parameter in determining the stability of a nanoparticle, other factors that also influence include size, distribution, and particle morphology [21].

3.3. Stability Test of BRY-E Nano-Capsule with Variation of Sonication Time

The method that is used in the making of nano-capsules is the sonication method. The usage of ultrasonic waves (sonication) in the formation of nano-sized materials is very effective. The given ultrasonic waves can break up the particles, previously particles formed bubbles that absorb the waves, then the particles break into smaller sizes [6]. Ultrasonic radiation breaks chemical bonds in three stages. The first stage is the formation of bubbles in the sonication bath. The second stage is bubble growth that occurs through solute vapor diffusion on the bubbles, and the third stage is bubbles burst that occurs when the size of the bubbles reaches its maximum value. Ultrasonic waves do not directly interact with molecules to induce a chemical change. The interaction of ultrasonic waves with molecules occurs through an intermediary in the form of a liquid. The waves generated by electric power are transmitted by the liquid medium to the intended target [7].

The characteristics of the BRY-E nano-capsules in the research that have been done include the level of turbidity and particle size. The level of turbidity can describe the stability of the nano-capsules formed. Table 4 shows the decrease in the turbidity value of each BRY-E nano-capsule. Turbidity was measured using a TDS (Total Dissolved Solids) measuring instrument equipped with a TDS sensor. The TDS sensor is the sensor that is used to measure the number of dissolved solids in a liquid in the form of organic ions, compounds, or colloids in water which is calculated in ppm (parts per million) or mg/L [22]. The results of pre-research that have been done at 24 hours of incubation showed that the nano-capsule formed was more stable than less than 24 hours.

Table 4. Turbidity value of BRY-E nano-capsulale

Sonication Time (minutes)	Duplication	TDS (mg/L)		Average		Increased / Decreased	Description
		0 hours	24 hours	0 hours	24 hours		
0	1	202	285	201.5	285.5	84 (increased)	a lot of
	2	201	286				
1	1	180	230	182	230.5	48.5 (increased)	
	2	184	231				
5	1	213	224	213	229	16 (increased)	
	2	213	234				
10	1	224	184	224	184	-40 (decreased)	precipitate
	2	224	184				
15	1	207	196	207	196	-11 (decreased)	precipitate
	2	207	196				

Based on data presented in Table 4, the treatment with a sonication time of 5 minutes was the best. In this condition, the smallest increase in turbidity was 16 NTU, it can be interpreted that 5 minutes sonication time resulting in the most stable BRY-E nano-capsule among the treatments given. At 10 minutes of sonication, turbidity levels consecutively decreased for 40 NTU and it was seen that a lot of nano-encapsulated BRY-E precipitated. This indicates that the formed nano-capsule is less stable. Table 5 shows the nano-capsule size in a variation of sonication time.

Table 5. Nano-encapsulated size in variation of sonication time

Sonication Time (minutes)	Duplication	Nano-capsule Size (nm)	PdI	Average of Nano-capsule Size (nm)	Average of PdI
5	1	825.1	0.385	890	0.401
	2	953.1	0.423		
	3	893.0	0.396		
10	1	2170	0.265	2146	0.345
	2	2096	0.423		
	3	2172	0.347		
15	1	3033	0.562	3047	0.522
	2	3010	0.549		
	3	3097	0.456		

Based on the data in Table 5, it can be interpreted that the sonication time affects the size of the nano-encapsulate produced. Sonication was done at a frequency of 30Hz that affected the particle breakdown, both in BRY-E and poloxamer matrix. The longer the sonication time, the higher the contact between the sample and the poloxamer matrix, so it has an impact on the coating or encapsulation of the matrix on the sample. Table 5 shows a sonication time of 5 minutes resulting in the smallest particle size among the treatments given.

4. CONCLUSION

The centrifugation carried out after sonication resulted in smaller particles compared to before sonication. The ratio 1:4 of BRY-E and the poloxamer matrix gave the best results, which produced particles with the size of 261 nm and zeta potential of -6.96. The best sonication time is 5 minutes, with a particle size of 890 nm. In terms of turbidity level, the best sonication time was 3 minutes that was marked by the smallest decrease in NTU during the storage period.

AUTHOR'S CONTRIBUTIONS

Rudiana Agustini: conceptualization and drafting manuscript. Nur Aida Amyliana: writing of method. Nuniek Herdiyastuti: Review and editing of manuscript; and I Gusti Made Sanjaya: data curation, data visualization and editing.

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