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NANOPARTICLE OF EGG YOLK IN FROZEN SEMEN DILUENT TO SUPPORT GERM PLASM CONSERVATION OF GARUT SHEEP

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ABSTRACT

The research purpose has been done to produce egg yolk freeze dry and egg yolk nanoparticles that quality in tris diluents to frozen semen storage to support germ plasm conservation of Garut ram. Specifically the objectives of the study can be formulated as follows: 1) Produce egg yolk freeze dry product through freeze drying process, 2) Minimize the size of the egg yolk through the nano-milling process, 3) Prove the potential of egg yolk freeze dry and egg yolk nanoparticles in tris diluents to maintain the quality of sperm resulting from storage of semen. The result of the research indicate fresh egg yolk, freeze dry egg yolk, commercial egg yolk, and milling egg yolk 110 have size particle and polydispersity index each of those are 13920.00±1065.55; 1.00±0.00, 1296.00±86.73; 0.70±0.04, 877.90±168.86; 0.34±0.04, and 403.06±7.05; 0.46±0.03. Egg yolk milling 110 with particle size (403.06±7.05 nm) has the smallest size when compared to fresh egg yolk, freeze dery egg yolk, adn commercial egg yolk. The potential zeta analysis of milling egg yolk 110 have good stability with zeta potential value is -16.68 ± 0.37 mV. The Zeta potensial test is only done on milling egg yolk 110. Morphology of freeze dry egg yolk and milling egg yolk 110 are almost the same, while commercial egg yolk is different. The conclusion of the research indicate milling egg yolk 110 has a smaller size compared to fresh egg yolk, freeze dry egg yolk, and commercial egg yolk. Milling egg yolk has good uniformity and good particle stability.

Keywords : freeze dry egg yolk, milling egg yolk 110, fresh egg yolk, commercial egg yolk, particle size and potential zeta

1. INTRODUCTION

1.1 Background

Indonesia is a country with tremendous amounts of genetic resources and biodiversity. Garut sheep is one of genetic resources and endemic indigenous ruminants of Indonesia as well as one of the excellent commodities that are potential to be preserved. Garut ram adult males have a relatively large weight compared to other local Indonesian ram, With an average weight of about 60 to 80 kg and can even reach 100 kg. In addition to having a large and vigorous posture, Garut ram has a good adaptability to the climate, a very good libido and a typical horn with a large size, strong, sturdy and circular, and commonly used as a ram fight. Garut ewe is one of the profilic trophical ewe because it can give birth to 1 to 5 lamb per birth. Among the people of West Java, Garut ram also has its own social and cultural values, thus Garut ram has higher economic value than other local ram and has great potential to develop. Garut ram can be used as semen donors with the aim of improving the appearance of other local ram through reproductive technology approach (Rizal *et al.* 2013).

Application of reproductive technology is one alternative to population increasement and genetic improvement. Various methods are developed continuously to increase the population and productivity of local ram such as artificial insemination (AI) technology. Processing of semen, especially in freezing process, is a technology that integrated with AI technology. The success of the AI program depends on various factors such as the quality of the semen used. Generally the quality of sperm will decrease in the freezing process, because of the extreme low temperature treatment. To prevent excessive sperm damage during freezing, various attempts can be made such as improving the quality of semen dilution material. Egg yolk nanoparticle is able to mix into tris diluent to maintain the quality of Garut ram freezing sperm. Through nanotechnology processing method of Garut ram semen diluent material, the number of semen straw that can be produced and also the number of ewe that can be inseminated will increase. Thus, research on semen diluent with nanometer size especially for Garut ram should be developed.

Tris yolk diluent is commonly used in Indonesia and even in some other countries as liquid semen diluents as well as frozen semen in ram. The result of freezing of Garut ram semen with tris egg yolk dilution is from 43.00% to 53.00% (Herdis *et al.* 2011). The frozen semen percentage of Garut ram semen was slightly lower compared to the result of freezing of ram in some other countries (52.00% to 62.90%) (Başpınar *et al.* 2011). Since the year 2000 some commercial companies has introduced lecithin-based diluent products that

already have sold in the market such as Biociphos (IMV, L'Aigle, France), Bioxcell (IMV, Technologies), Andromed (minitub, Germany), Botu-Semen dan Botu-Cryo (Botupharma, Brazil). Although these commercial products have been used, but the dilution of these diluents do not completely replace the popularity of egg yolk diluent.

Egg yolk is an important component commonly added to semen diluents and has been recognized to protect sperm during the freezing process. Lecithin, phospholipids and lipoprotein fractions are the components of egg yolk as cryoprotective agents (Vishwanath and Shannon 2000; Aboagla and Terada 2004). These component are able to bind or envelop the membrane plasm to maintain the stability of the sperm (Botham and Mayes 2009; Ricker *et al.* 2006; Moussa *et al.* 2002). The addition of the egg yolk in the diluent also has a property as a buffer of osmotic pressure so that sperm cells are more tolerant to hypotonic and hypertonic diluents (Khalifa and El-Saidy 2006) and prevent of calcium flow increasement into the cells that can damage the sperm (Purwoistri *et al.* 2013). The advantages of egg yolk as sperm diluent are source of sperm energy, membrane plasm and acrosome protector againts cold shock, especially when combined with other components during storage before artificial insemination (Amirat *et al.* 2004). Low density lipoprotein (LDL) is thought to play an active role in protecting the membrane plasm of sperm during freezing (Moussa *et al.* 2002). LDL composes about 2/3 of the total solids of chicken egg yolks. LDL is considered a contributor to the cryoprotective nature of egg yolks that are spherical particles with triglyceride nuclei and cholesterol esters that surround the apoprotein and phospholipid layers (Pillet *et al.* 2011).

Egg yolks that having a particle size of 13920.00±1065.55 nm commonly uses in semen diluent will only provide the protection from outside the membrane plasm of sperm. With nanotechnology method, egg yolk particle size can be reduced to the egg yolk particles below 1000 nm or a range of 0.1 nm to below 1000 nm. Smaller particle size of egg yolk is expected will increase the number of egg yolk particle that will diffuse through membrane plasm and maintain the semen quality after thawing. In Indonesia, nanotechnology methods have been widely applied to the manufacture of herbal medicines such as nano-propolis, temulawak and ginger, food products such as milk, and cosmetic manufacturing. Therefore, it is possible to apply the egg yolk as a semen diluent. The principle of nanotechnology is to reduce the size of large particles to particles size below 100 nm or 0.2 nm to 100 nm range without changing the properties of the material. In simple terms, the size of 1 nm is one per one billion meters (10^{-9}) which means 50000 times smaller than the size of a human hair (Lankalapalli et al 2014). According to Farokhzad and Langer (2009), particles in nanometer

sizes are 0.1 nm to below 1000 nm or less than 1 µm. The biological activity of nanoparticles is tens of times higher than macroscopic particles (Salah *et al.* 2011). The manufacture of semen diluent on a nanometer scale is a new strategy in developing the method of making semen diluent. Using nanometer-sized particles added to the semen diluent, the nanoparticles more easily diffuse into the sperm cells (Odhiambo *et al.* 2014). Further Odhiambo *et al.* (2014) reported that the addition of Fe_2O_3 nanoparticles conjugated with ubiquitin and PNA lectins into the semen diluent can be increase the conception rate due to abnormal sperm removal in the semen mixed into the diluent.

Nanotechnology development strategy should be done to increase the value of egg yolk as livestock semen diluent, especially Garut ram, and can be useful to develop the potential of sheep as germ plasm of Indonesia. Beside use on Garut ram, for the conservation purpose of native germ plasm of Indonesia, nanotechnology method in the manufacture of egg yolk nanoparticle also has be done on other animals such as Sumatran tiger (Karja *et al.* 2016), Rawa buffalo (Rizal and Riyadhi 2016), Lumpur buffalo (Rosadi *et al.* 2015), Bali cattle, Madura cattle, and Ongole Crossbreed cattle (Salim *et al.* 2012), Etawah Crossbreed or Randu Java goat (Ariantie *et al.* 2013), anoa (Yudi *et al.* 2010), and Sumatran rhinoceros (Agil *et al.* 2002).

1.2 Research Purposes

The general objective of the study has been to produce egg yolk freeze dry and egg yolk nanoparticles that quality in tris diluents to frozen semen storage to support germ plasm conservation of Garut ram. Specifically the objectives of the study can be formulated as follows:

1. Produce egg yolk freeze dry product through freeze drying process.
2. Minimize the size of the egg yolk through the nano-milling process.
3. Prove the potential of egg yolk freeze dry and egg yolk nanoparticles in tris diluents to maintain the quality of sperm resulting from storage of semen.

1.3 Expected Outcome

This research has been conducted with the expectation among others as follows:

1. Obtained egg yolk freeze dry and egg yolk nanoparticles as semen diluent to maintain the quality of freezing sperm.
2. Provides information on the effect of egg yolk freeze dry and egg yolk nanoparticles on the storage of frozen semen.

3. Provide recommendations on nanometer-sized diluents made in the country that can be used for the conservation of native germ plasm of Indonesia

2. BENEFITS OF RESEARCH AND IMPORTANCE OF IMPLEMENTATION

a. Benefits of Research

This research is expected to provide academic benefits in the field of science and research, and practically for for related institutions. Academic benefits include producing egg yolk freeze dry and egg yolk nanoparticles on diluents especially tris which can be utilized for storage of frozen semen, and as the conservation of native germ plasm of Indonesia by maintaining a higher quality of freezing sperm than regular egg yolks. The benefits for the related institutions are providing recommendations to research centers and artificial insemination centers and Conservationists concerning freeze dry products and egg yolk nanoparticles as high quality semen diluents to support the Indonesian germ plasm conservation program.

b. Importance of Implementation Research

Research on egg yolk freeze dry using freeze drying method and egg yolk nanoparticle using milling modification method as a diluent (especially tris) used in storage of frozen semen was first performed in Indonesia and still very rare in the world. Thus, it is necessary to make innovations in the field of storage of frozen semen and the implementation of nanotechnology for the manufacture of egg yolk nanoparticles as a frozen semen diluent is essential for the successful application of AI. Therefore, this study is very important to do and as a reference in the use of nanometer-sized egg yolk in semen diluents. Nanometer-sized egg yolk in semen diluents that are expected to diffuse into sperm cell membran so as to obtain the superior quality of sperm to support the conservation of native Indonesian animals.

3. METHODOLOGY

a. Research Locations

Egg yolk preparation, egg yolk drying and egg yolk nanoparticles preparation, semen collection, semen preservation and cryopreservation has been done in Reproductive Rehabilitation Unit laboratory, Division of Veterinary Reproduction, Obstetric and Gynecology, Faculty of Veterinary Medicine, Bogor Agricultural University. Frozen semen

analysis has been done Reproduction, Genetics and Animal Cell Culture laboratory, Research Center for Biotechnology, Indonesian Institute of Sciences, Cibinong. Nanoparticles morphology has been done Nanotechnology laboratory, Indonesian Agency for Agricultural Research and Development Ministry Agriculture, Bogor.

b. Animal Experiments

Experimental animals used has been treated in accordance of the ethics of the research. Animals that has been used as the source of semen are 4 rams superior male in the age group 4 to 6 years with a range of 60 kg to 80 kg body weight. Livestock will place in individual cages equipped with food and drink container. The feed that has been given are 20% fresh elephant grass forage per body weight and 2% concentrate per body weight by giving twice a day, and drinking water has been provided ad libitum. Males that has been used in this research are ram that have a good semen quality with some criteria such as have more than 70% motility of sperm, concentration is greater than $2000x10^6$ per mL and less than 15% abnormalities of sperm.

c. Experimental Procedure

The research has been conducted in three parts, such as:

1. Freeze dry egg yolk production using freeze drying method
2. Egg yolk nanoparticle production using milling modified method
3. Storage and effect of the quality of frozen semen of Garut ram using tris-freeze dry egg yolk and tris-egg yolk nanoparticle diluent

3.1 Freeze dry egg yolk production using freeze drying method

a. Egg Yolk Preparation

Egg yolk plasma preparation that has been used is placed on the filter paper and egg yolk collected in a measuring cup, while the egg albumin discarded. Egg yolk collection 1000 mL, and the egg yolk are frozen at -5 ◦C for 24 hour.

Chicken eggs used are Lohmann chicken eggs with a age range of 56-84 weeks from Global Buawana Farm, which is located in Cihideung Udik, Ciampea, Bogor, owned by Mr Gerry Buawana.

b. Freeze Drying Egg Yolk

The drying process of egg yolk is done using a freeze dryer machine (Christ®, Gamma 1-20) for 144 hours at a temperature -40 ◦C and a vacuum pressure of 1.030 – 0.630 mbar. The dried egg yolk stored at a temperature -4 ◦C.

3.2 Egg yolk nanoparticle production using milling modified method

a. Preparation Processes of Egg Yolk Nanoparticle

The method of preparation processes of egg yolk nanoparticle modifies the method performed by Ikono *et al*. 2012, the stages are egg yolk samples dissolved with *aquades* into milling chamber with speed 10000-20000 rpm at temperature 4 ◦C. The destruction process is done for 110 minute. Particle size analysis has been measured by dynamic light scatering (DLS) using Nanosizer Malvern Instruments (Malvern, England).

b. Particle Size Analysis

Particle size analysis of egg yolk has been measured by dynamic light scatering (DLS) using Nanosizer Malvern Instruments (Malvern, England). Samples are stored into 1 cm plastic cell spectroscopy and then transferred to Nanosizer is set at 20 °C. For each sample, diameter was measured third. Each measurement is the diameter corresponding to five autocorrelation function accounted for 20 seconds (Pillet et al. 2011).

According to Schubert and Müller-Goymann (2005) particle size measurement using laser diffraction is used to determine of particle size and particle size distribution of the system with an average particle size above 1000 nm. Measurements has been performed with the Mastersizer MS 20 (Malvern, Herrenberg, Germany). Before measurement, about 2 mg of sample was diluted with 10 mL of milli-Q. Results are calculated by the software using the Malvern SB 09 Fraunhofer approach.

Formulations with an average particle size of the particles are smaller than 1000 nm was observed by Photon Correlation Spectroscopy (PCS) using a Zetasizer 3 (Malvern, Herrenberg, Germany). The particle size (z average) and particle size distribution (polydispersity index) are calculated by the software using the Malvern SB 09. The average z diameter of the particles used to estimate the area surface specifik (Aspec) of sample assuming a spherical shaped particle shape and density of 1 g / cm^3:

$$\text{Aspec} = \frac{\text{surface area}}{\text{density x volume}} = \frac{4\pi r^2}{\rho 4/3\pi r^3} = \frac{3}{\rho \text{ x } r}$$

c. Potential Zeta Measurement

Electrophoretic mobility is determined by laser Doppler anemometry (LDA) in mikroelektroforesis AZA cells from a Zetasizer 3 (Malvern, Herrenberg, Germany). This instrument is operated in a mode cross beam modulation frequency of 1000 Hz and a voltage of 150 V. Each measurement is performed three repetitions (Schubert and Müller-Goymann 2005).

d. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (JSM-5600 LV, JEOL USA) was used to determine the shape and surface morphology of nanoparticles produced. SEM requires samples with platinum coatings in a Aauto Fine Coater (JFC-1300, JEOL USA). AFM has been conducted with Nanoscope IIIa in tapping mode. Nanoparticle samples has been mounted on a metal plate using double-sided adhesive tape and scanned by the AFM at a constant temperature and vibration-free environment (Mu and Feng 2003).

3.3. Storage and effect of the quality of frozen semen of Garut ram using tris-freeze dry egg yolk and tris-egg yolk nanoparticle diluent

a. Diluent Medium Preparation.

Basic Medium that has been used in the study is tris-buffer diluent base. Tris buffer contains 2.98 g tris-hydroxymethyl-aminomethane, 1.65 g citric acid monohydrate and 2 g D-fructose. The material is dissolved in 100 mL of milli-Q water, then added with egg yolk nanoparticle, egg yolk freeze dry, commercial egg yolk and fresh egg yolk. After that, the diluent is distilled at a speed of 500 rpm to be homogeneous. The solution has been centrifuged for 30 minutes at a speed of 3000 rpm. Each diluent has been added with 4% glycerol cryoprotectant (v/v). Then added streptomycin 1 mg (Meiji, Japan) and penicillin 1000 IU ml-1 (Meiji, Japan). The combination of tris buffers with egg yolk nanoparticle, egg yolk freeze dry, egg yolk commercial and egg yolk has been repeated 3 times. The treatments that has been conducted are:

1. Tris buffer + 20% egg yolk nanoparticle
2. Tris buffer + 20% *freeze dry* egg yolk
3. Tris buffer + 20% commercial egg yolk
4. Tris buffer + 20% fresh egg yolk (control)

b. Colection and Evaluation

Semen Collection. Semen collection has been using a vagina artificial once a week with 3 times holding each consisting of two of the ejaculate (two times in one period). Semen that has been successfully collected directly stored in water bath (32 ^{o}C) until evaluation.

Semen Evaluation. Semen evaluation has been done macroscopically and microscopically. Macroscopic evaluation includes the volume, pH, consistency and color.

- Volume, determined by observing on a measuring pipette of 0 to 5 mL.
- Potential Hidrogen (pH), measured using a pH indicator paper 6.4 to 8.0.
- Consistency, evaluated by on the speed of the semen back to the bottom of the container tube. Criteria for condensed and moderate assessment.
- Colour, evalauted and divided into creamy and white.

Microscopic evaluation includes mass movement, progressive motility, individual movement, concentration, viability sperm, morphology, plasma membrane intact (PMI) and acrosome integrity.

- Mass movement has been done by making preparations by putting a drop of fresh semen on top of the object glass and observed using a microscope with magnification 10 x 10. The assessment is done by looking at the thickness of the mass waves of sperm and the speed of the sperm waves move. The classification of assessment criteria is +++ (heavy and rapidly moving mass waves), ++ (thick but slow moving mass wave or medium mass but fast switching mass), + (thin and slow mass wave displacement), and - (no mass wave).
- Motility of sperm and individual movement has been observed under light microscope with 10x40 magnification and computer assisted sperm analyzed (CASA), that is Spermvission® 3.7 (Minitube, Germany).. CASA can assess the movement of spermatozoa, (progressive motility spermatozoa). Evaluation is done with first diluting the semen using a 0.9% physiological NaCl. The motility of sperm is assessed by estimation from 5 fields of view by comparing the number of progresive motility with other sperm movements (dead sperm, vibrating in place, spinning and moving backwards). Values are expressed in percent. While the criteria for scoring speed scores to move forward is 0 to 5. The classification of assessment criteria is 0 (no advanced movement of individual sperma), 1 (advanced individual movements of slow sperm), 2 (advanced individual movements of moderate sperm), 4 (advanced individual movements of rapid sperm), and 5 (advanced movement of individual sperm very quickly).

- Concentration of sperm has been done using Neubauer count chamber. The sement has been diluted using a formolsaline solution 1: 500 (2 µL semen : 998 µL *formolsaline*) and homogenized in microtube. Calculations of sperm has been performed on 5 large squares of a total of 25 boxes in the Neubauer count chamber. Each big box consists of 16 small squares and all the sperm inside are counted. Sperm whose heads are in the box are counted 1, while whose head is at the line boundary is calculated 1/2. The results of the calculation of sperm from both count rooms then averaged (N = N1 + N2 / 2). Concentration of sperm has been calculated using the following formula:

  $$\text{Total of sperm ml}^{-1} = N \times 5 \times 500 \times 10000$$

  Number 5 is a correction factor in which the count is only performed on 5 rooms out of a total of 25 rooms (25/5 = 5). Dilution factors used are 500 and 10.000 is a necessary correction factor because the cover slip depth is 0.0001 mL per chamber.
- Viability of spem, evaluated using eosin-nigrosin staining. One drop of semen is added with ten drops of eosin nigrosin dye homogenised on a glass object, preparing semen smear and dried over a heating table. The preparation of the preparations is done quickly. At least 200 cell sperm has been calculated based on the calculation from approximately 10 visual field using light microscope with 10x40 magnification. Dead sperm absorb the color, while the living does not absorb the color (transparant).
- Abnormality of sperm evaluated using Williams staining using carbol-fuchsin, by first diluting the cement using 0.9% physiological NaCl, then make a semen smear done in a short time, then dried. Coloring is done by fixing the semen smear by washing in absolute alcohol for 4 minutes, then dried. Then put into 0.5% chloramine solution for 1 to 2 minutes, While in the lift and re-input repeatedly with the aim of eliminating mukus until the preparations look clear. Wash in distilled water then put into 95% alcohol. Then stained with Williams staining solution for 8-10 minutes. semen smear washed with running water until the water looks clear, then dried. At least 200 cell sperm has been calculated based on the calculation from approximately 10 visual field using light microscope with 10x40 magnification. Assessment of abnormalities performed on primary, secondary and tertiary abnormalities.
- The plasma membrane integrity (PMI), adopt from Fonseca *et al*. (2005) by dissolving 10 µL of semen into 1000 µL hypoosmotic swelling test (HOS-test) solution, then incubated at 37 ◦C for 30 minutes. Composition of the hypoosmotic swelling test solution: 7.35 g sodium citrate, 13.52 g fructose dissolved in 1000 mL of water distillet then stirred; osmolarity 150 mOsm. At least 200 cell sperm has been calculated based on the

calculation from approximately 10 visual field using a 10x40 magnification light microscope. The PMI is characterized by a circular sperm tail, while the damaged one is marked by a straight tail.

- The acrosome integrity is evaluated by diluting of the semen by using formolsaline solution to fixing of sperm. Sperm that still have an acrosome integrity is marked with 1/2 to 2/3 of the anterior head is darker than the posterior. At least 200 cell sperm has been calculated based on the calculation from approximately 10 visual field using phase contrast microscope with 10x100 magnification. The acrosome integrity has been divided into four categories. They are normal apical ridge (NAR), damaged apical ridge (DAR), missing apical ridge (MAR) and loose acrosomal cap (LAC) (Yi *et al.* 2008).

c. Semen Cryopreservation

Specification of frozen semen quality are having more than 70% progressive motility of sperm, concentration is greater than 1000×10^6 per mL and less than 15% abnormalities of sperm. Semen which have a good quality has been processed to frozen semen.

Freezing methods has been performed in one stage. Freezing semen has been prepared with 100×10^6 per 0.25 mL insemination dose. Then, straw containing semen has been equlibrated in a refrigerator at a temperature of 5 °C for four hours. After equlibration, straw that contain semen has been placed on the rack freezing with a distance of 3 cm above the surface of the liquid nitrogen vapor (N_2) for 10 minutes (–120 °C) in Styrofoam box. The frozen semen will stored in liquid N2 containers (-191 - -192 °C) to be evaluated.

The quality of freezing semen is determined progressive motility, percentage of live, plasma membrane integrity (PMI), and acrosome integrity after thawing in 37°C for 30 seconds. The volume of diluent is calculated by artificial insemination dose.

The success of freezing also has been assessed from recovery rate (RR). Recovery rate is the number of sperm that successfully recovered from the freezing process and calculated according to Garner and Hafez (2000) with the formula:

$$R = \frac{\text{Percentage of progressive motility after thawing}}{\text{Percentage of progressive motility fresh semen}} \times 100\ \%$$

3.4. Research Design and Data Analysis

The research design has been used Completely Randomized Design. Research data has been used Analysis of Variance (ANOVA), if there was any significant difference between treatment then continued with Duncan test. Data processed using SPSS version 22 program.

4. RESULTS AND DISCUSSION

4.1. Particle size and polydispersity index of egg yolk

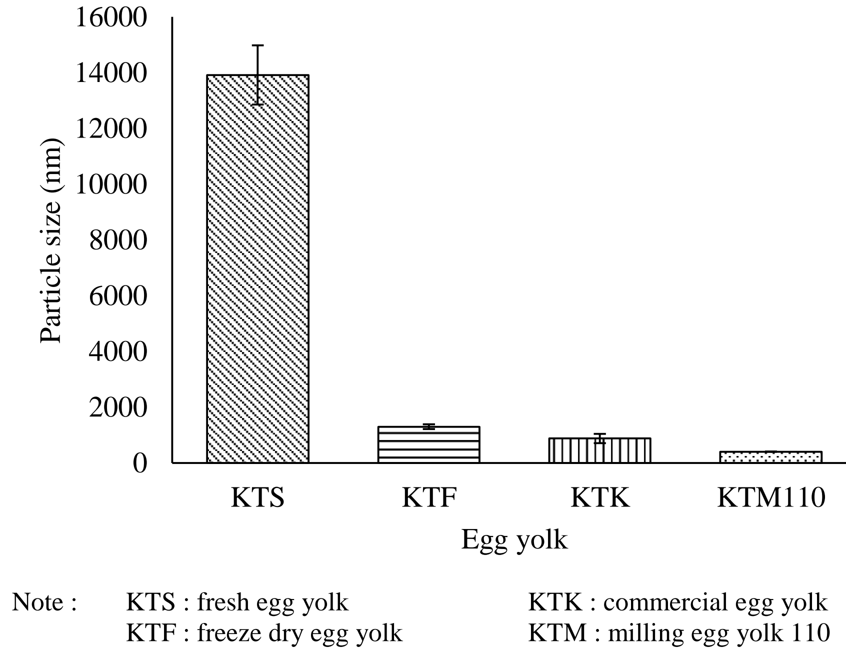


Figure 1. Particle size of fresh egg yolk, freeze dry egg yolk, commercial egg yolk and milling egg yolk 110

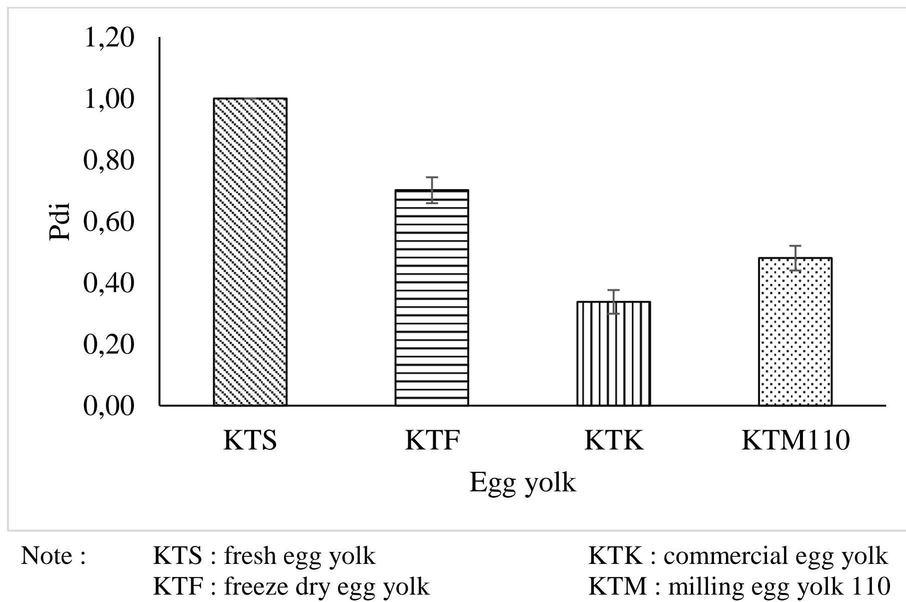


Figure 2. Polydispersity index (Pdi) of fresh egg yolk, freeze dry egg yolk, commercial egg yolk and milling egg yolk 110

Table 1. Particle size and Polydispersity index (Pdi) of fresh egg yolk, freeze dry egg yolk, commercial egg yolk and milling egg yolk 110

Treatment	Particle size (nm)	Pdi
Fresh egg yolk	13920.00±1065.55	1.00±0.00
Freeze dry egg yolk	1296.00±86.73	0.70±0.04
Commercial egg yolk	877.90±168.86	0.34±0.04
Milling egg yolk 110	403.06±7.05	0.46±0.03

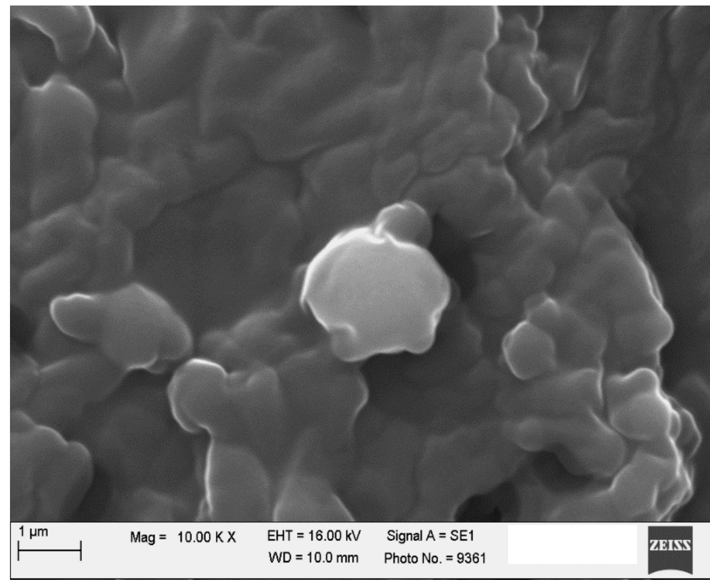


Figure 3. Morphology of freeze dry egg yolk

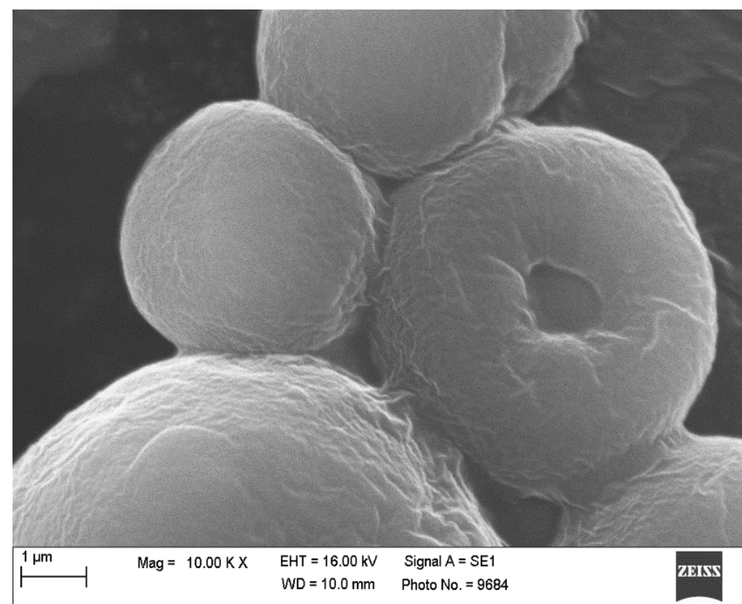


Figure 4. Morphology of commercial egg yolk

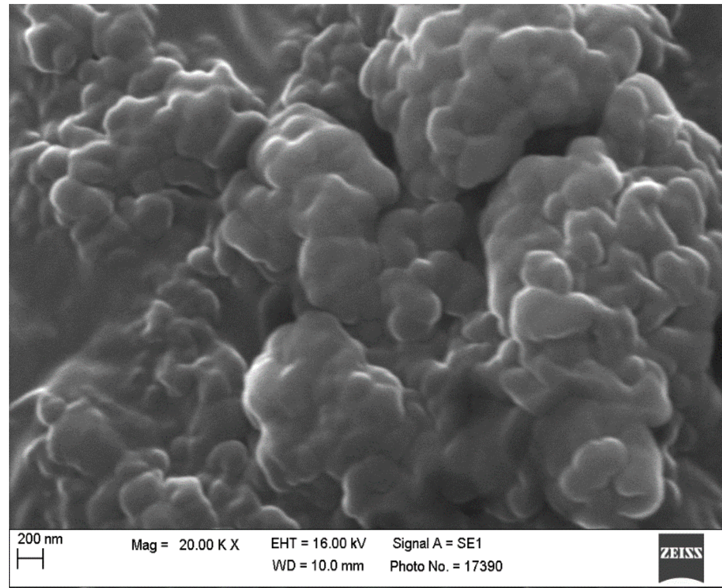
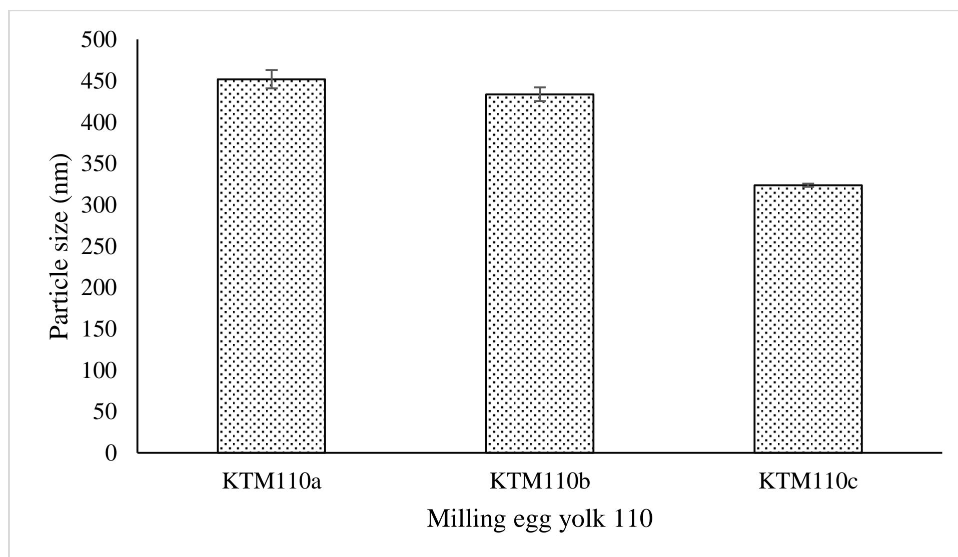


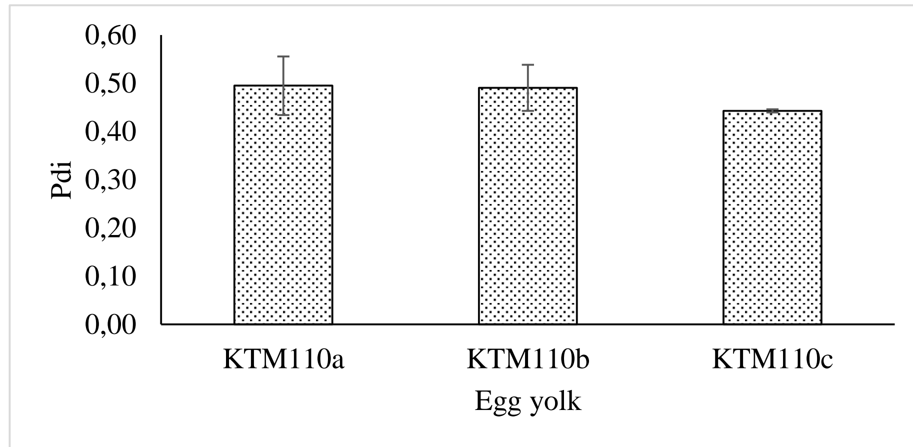
Figure 5. Morphology of milling egg yolk 110

4.2. Particle size, polydispersity index and zeta potential of milling egg yolk 110



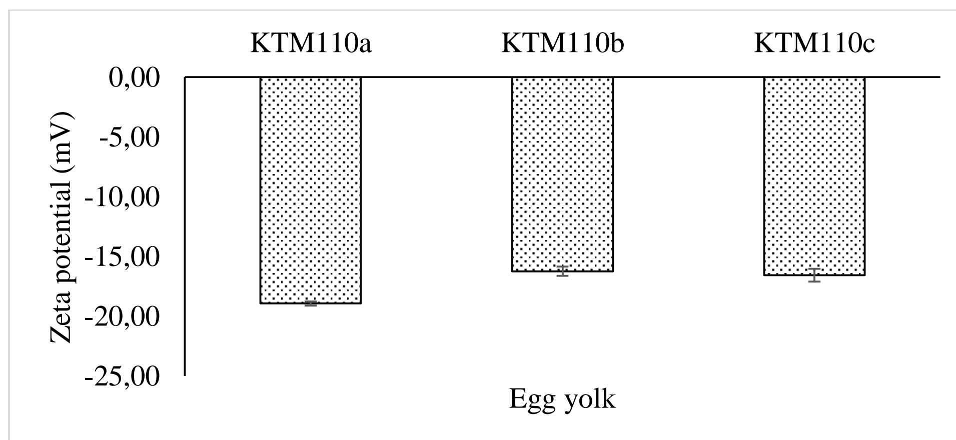
Note : KTM110a : milling egg yolk 110a
KTM110b : milling egg yolk 110b
KTM110c : milling egg yolk 110c

Figure 6. Particle size of milling egg yolk 110



Note : KTM110a : milling egg yolk 110a
KTM110b : milling egg yolk 110b
KTM110c : milling egg yolk 110c

Figure 7. Polydispersity index of milling egg yolk 110



Note : KTM110a : milling egg yolk 110a
KTM110b : milling egg yolk 110b
KTM110c : milling egg yolk 110c

Figure 8. Zeta potential of milling egg yolk 110

Table 2. Particle size, polydispersity index (Pdi), and zeta potential of millling egg yolk 110

Treatment	**Particle size (nm)**	**Polydispersity index (Pdi)**	**Zeta Potential (mV)**
Milling egg yolk 110a	451.87±11.00	0.49±0.06	-18.93±0.17
Milling egg yolk 110b	433.70±8.29	0.49±0.05	-16.23±0.38
Milling egg yolk 110a	323.60±1.87	0.44±0.00	-16.57±0.52
Mean	**403.06±7.05**	**0.46±0.03**	**-16.68±0.37**

In this study, fresh egg yolk, freeze dry egg yolk, commercial egg yolk, and milling egg yolk 110 have size particle and polydispersity index each of those are 13920.00±1065.55; 1.00±0.00, 1296.00±86.73; 0.70±0.04, 877.90±168.86; 0.34±0.04, and 403.06±7.05; 0.46±0.03 (Table 1 and 2). Egg yolk milling 110 with particle size (403.06±7.05 nm) has the smallest size when compared to fresh egg yolk, freeze dery egg yolk, adn commercial egg yolk (Figure 1 and 2).

The analysis of the egg yolk has a nanometer size (403.06±7.05 nm) indicates good stability (Figure 6). Some of sources mentioned nanoparticles will indicatestheir characteristics at sizes below 100 nm, but this limit is difficult to achieve. however, it was agreed that nanoparticles are particles that have a size below 1 micron or 1000 nm (Tiyaboonchai 2003; Buzea *et al.* 2007), but sizes below 500 nm have better characteristics.

Polydispersity index value (0.46±0.03) indicates uniform particle size (Figure 7). According to, Yuan *et al.* 2008, the smaller the polydispersity index value, the particle size is more homogeneous. Avadi *et al.* 2010, Pdi value greater than 0.5 indicates high heterogeneity, and if approaching the value 0 indicates a uniform size.

Nanoparticle synthesis that makes smaller of particle size (nanometer-sized) can be increase the surface area of the particles so that the permeability of the cell membrane increases, this can result in the use of egg yolk nanoparticles to maintain the viability of spermatozoa. According to Salah *et al* 2011, the biological activity of nanoparticles is tens of times higher than macroscopic particles.

Some the advantages of nanoparticles are the ability to penetrate the cell surface which can only be penetrated by nanometer size (Buzea *et al.* 2007). Ability to penetrate higher cell membrane, both through diffusion or opsonification. Nanoparticle have high flexibility to be combined with other technologies so it’s potential for developed for various purpose and targets. Another advantages of nanoparticles is their presence increased affinity (Kawashima 2000).

Zeta potential of milling egg yolk 110 have good stability with zeta potential value is -16.68 ± 0.37 mV (Table 2 and Figure 8). The Zeta potensial test is only done on milling egg yolk 110. Nanoparticles with zeta potential values smaller than -30 mV and greater than +30 mV has higher stability (Murdock *et al.* 2008). Dispersion system with low zeta potential value is easier to form aggregates in particle interactions (Nanocomposix 2012).

The zeta potential is usually used for characterize electric charges of surface nanoparticles, related to electrostatic nanoparticles interactions. Electrostatic interactions

will determine the tendency aggregation and reject refuse. Zeta potential is the size of the particle's surface charge spread in dispersing medium.

Morphology of Freeze dry egg yolk and milling egg yolk 110 are almost the same (Figure 3 and 5), while commercial egg yolk is different (Figure 4). Observation of egg yolk morphology tends to be uniform. But if measured polydispersity index value of freeze dry egg yolk with polydispersity index value 0.70±0.04 indicates non-uniform particles. While, commercial egg yolk and milling egg yolk with polydispersity index values of 0.34±0.04 and 0.46±0.03 show uniform particles. Avadi *et al.* 2010, Pdi value greater than 0.5 indicates high heterogeneity, and if approaching the value 0 indicates a uniform size.

5. CONCLUSION

The milling egg yokl 110 has a smaller size compared to fresh egg yolk, freeze dry egg yolk, and commercial egg yolk. Milling egg yolk has good uniformity and good particle stability.

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