

# Effect of rice variety and reaction parameters on synthesis and antibacterial activity of silver nanoparticles

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## Summary

In the present study, three different rice varieties; Jasmine (JM), Niaw Koko-6 (NKK), and Saohai (SH) were determined for reducing power using ferric reducing antioxidant power (FRAP) assay. SH showed the highest reducing property followed by JM and NKK, respectively. All modified rice samples were used to fabricate silver nanoparticles (AgNPs) by reducing silver nitrate (AgNO<sub>3</sub>) to metallic Ag. The obtained AgNPs from JM, NKK, and SH namely JM-AgNPs, NKK-AgNPs, and SH-AgNPs, respectively, showed maximum absorption at 410, 408, and 409 nm, respectively, which confirmed the spectra of AgNPs. Reaction parameters such as AgNO<sub>3</sub> and modified rice concentration as well as the reaction period were investigated. It was found that increasing of these parameters gave better AgNPs until the concentration of modified rice and AgNO<sub>3</sub> reached to 0.3% and 10 mM, respectively and the reaction period reached to 60 min, the most suitable AgNPs were obtained. Among the three rice varieties, SH showed the most potential for synthesis of AgNPs. SH-AgNPs showed the smallest size of 80.4 ± 2.8 nm and the highest zeta potential of -45.9 ± 1.4 mV. The AgNPs obtained from all three rice varieties showed effective against *Escherichia coli* than *Staphylococcus aureus* and SH-AgNPs showed significantly higher antibacterial activity than JM-AgNPs and NKK-AgNPs.

**Keywords:** AgNPs, modified rice, rice variety, green synthesis, antibacterial activity

## 1. Introduction

Silver nanoparticles (AgNPs) have been increasing interested in medical applications because of their ability to inhibit many important pathogenic bacterial such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (1) owing to the effects of AgNPs on bacterial membrane (2). Moreover, AgNPs have shown anti-inflammatory (3) and anticancer (4) activities. Previously, AgNPs were prepared by using chemical reaction between silver nitrate (AgNO<sub>3</sub>) and chemical reducing agents, such as hydrazine hydrate, sodium citrate, and tannic acid (5,6) to reduce Ag<sup>+</sup> to metallic silver (Ag<sup>0</sup>). This may increase chemical

waste to environment. Nowadays, there are many efforts to reduce generated hazardous waste by using active components from certain potential plants having reducing property, such as *Mentha piperita* (7), *Psidium guajava* (8), and *Cymbopogon citratus* (9).

Rice is the principle food for people in many countries. We previously reported that rice extract has antioxidant and reducing properties (10). However, the concentration of the modified rice used previously was too low to show significant reducing property. In the present higher rice concentration was used with the main aim of using modified rice as a reducing agent in the preparation of AgNPs (Rice-AgNPs). The effects of rice variety and rice concentration as well as other reaction parameters such as AgNO<sub>3</sub> concentration and time of reaction were investigated. The obtained rice-AgNPs were characterized by UV-vis, Fourier transmission infrared (FTIR) spectroscopy, photon correlation spectrophotometry (PCS). The inhibitory activity of

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the rice-AgNPs to Gram positive and Gram negative bacteria was evaluated by measuring the inhibition zones under well diffusion method and determining minimum inhibition concentration (MIC) as well as minimum bactericidal concentration (MBC) using micro-dilution method.

## 2. Materials and Methods

### 2.1. Materials

Milled rice grains of three rice varieties including JM, NKK, and SH were obtained from a local market in Chiang Mai, Thailand. AgNO<sub>3</sub>, methanol, and glacial acetic acid were supplied by RCI Lab-scan Co., Ltd. (Bangkok, Thailand). Potassium bromide (KBr) for FTIR was purchased from Fisher Scientific, (Loughborough, UK). Tryptic soy agar (TSA) and broth (TSB) were supplied by Difco™ (Baltimore, Maryland, USA). All other chemicals and solvents were of AR grade or the highest grade available.

### 2.2. Bacterial strains

The aerobic bacterial strains of *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 represented for Gram-positive and Gram-negative bacteria, respectively, were used.

### 2.3. FRAP assay

Three rice grains of JM, NKK, and SH were chemical modified according to the method previously described by Okonogi *et al.* (11). The reducing power of the modified rice was determined using FRAP assay according to the method described previously (12) with some modification. Briefly, the FRAP reagent was freshly prepared by mixing 2.5 mL of 10 mM TPTZ solution in 40 mM HCl with 2.5 mL of 20 mM FeCl<sub>3</sub> and 25 mL of 0.3 M acetate buffer, pH 3.6. An amount of 20 µL dispersion of modified rice samples were mixed with 120 µL of FRAP reagent in 96 well plate. Blank samples were prepared by mixing acetate buffer and different concentration of JM, NKK, and SH. The samples and blank were incubated for 10 min at room temperature and the absorbance of the samples was determined at 595 nm using microplate reader (Bio-Rad, Model 680, Hercules, California, USA). The reducing power of the samples was evaluated by calculating the amount of Fe<sup>2+</sup> produced by modified rice starch samples using the calibration curve of FeSO<sub>4</sub>. All data were run in triplicate.

### 2.4. Synthesis of AgNPs

#### 2.4.1. Effects of reactant concentration

Aqueous solutions containing 0.1, 0.2, 0.3, 0.6, and 0.9 % w/v of modified rice were prepared. In the same time,

aqueous solutions of 2.5, 5, and 10 mM AgNO<sub>3</sub> were separately prepared. Then, add AgNO<sub>3</sub> solution dropwise to the rice solution at 75°C with continuous stirring until the volume ratio of the rice solution and AgNO<sub>3</sub> solution was 100:1. The reaction was kept at this temperature under continuous stirring for 60 min. The obtained rice-AgNPs were cooled down to room temperature for further studies.

#### 2.4.2. Effects of reaction period

An aqueous solution containing 0.3 % w/v of modified rice was prepared. Then, 10 mM AgNO<sub>3</sub> solution was added dropwise to the rice solution at 75°C with continuous stirring until the volume ratio of the rice solution and AgNO<sub>3</sub> solution was 100:1. The reaction was kept at this temperature under continuous stirring for 15, 30, 60, and 90 min. The obtained rice-AgNPs were cooled down to room temperature for further studies.

### 2.5. Characterization of AgNPs

#### 2.5.1. UV-Vis

The rice-AgNPs obtained from each preparation condition was diluted to 100 fold with deionized water. The outer color appearance of the rice-AgNPs was observed by visualization. The optical property of the rice-AgNPs solution was observed using UV-Vis spectrophotometer (Shimadzu-2450, Kyoto, Japan) in the wavelength range of 200-700 nm.

#### 2.5.2. PCS

The size, size distribution (PDI), and zeta potential of rice-AgNPs were investigated using PCS (Malvern Zetasizer Nano ZS, Malvern instrument, Worcestershire, UK) at 25°C. Each sample was diluted to 100 fold with deionized water before measuring.

#### 2.5.3. FTIR

The lyophilized rice-AgNPs and the modified JM, NKK, and SH in powder form were subjected to FTIR in order to investigate for functional group spectra. The samples were prepared in a KBr disc. The FTIR spectra of the samples were recorded in the range of 4,000-400 cm<sup>-1</sup> using a smart diffuse reflectance, Nicolet Nexus 470 FT-IR (Minneapolis, Minnesota, USA) in the diffuse reflectance mode at a resolution of 4 cm<sup>-1</sup> at room temperature. The spectra were collected against a KBr disc background at a controlled ambient temperature at 25°C.

### 2.6. Evaluation of antimicrobial activity

#### 2.6.1. Well diffusion method

A well diffusion method for evaluation of antibacterial activity against the test pathogenic bacteria of the obtained JM-AgNPs, NKK-AgNPs, and SH-AgNPs was based on Kirby-Bauer method (13). The test strains of *S. aureus* and *E. coli* were grown in TSA at 37°C for 24 h. Then, they were diluted in TSB to a final density of  $1.5 \times 10^6$  colony-forming units (CFU)/mL. The density of the microbial suspension was adjusted with 0.5 McFarland constant by observing the wavelength at 600 nm in a UV-vis spectrophotometer. The bacterial suspensions were swabbed on the agar surface by using sterile cotton swab. Aqueous mixtures (40  $\mu$ L) of JM-AgNPs, NKK-AgNPs, and SH-AgNPs were added onto the wells in agar plates, the diameter of well was 6 mm. The plate were incubated at 37°C for 24 h. The antimicrobial activity was evaluated by determining the diameter of the clear zone of inhibition around the well expressed in millimeter (mm). All samples were done in triplicate.

### 2.6.2. Broth dilution method

Aqueous solutions containing 0.1 mg/mL of lyophilized rice-AgNPs were prepared in deionized water and diluted in TSB containing the test bacteria. The results were evaluated after 24 h of incubation at 37°C. The minimum rice-AgNPs concentration giving the clear solution in this step denoted the MIC. For MBC determination, the clear samples resulted from the MIC series were swabbed on the agar plates as indicated by Clinical and Laboratory Standards Institute (CLSI) guideline (14). The minimum rice-AgNPs concentration showing no bacterial growth in agar plate denoted the MBC. All samples were done in triplicate.

### 2.7. Statistical analysis

Data were analyzed using a One-way analysis of variance (ANOVA) and Duncan's multiple range test statistic a software version 17 (SPSS Inc., Chicago, United States). The values were presented as means  $\pm$  standard deviation which a *p*-value less than 0.05 was considered as a significant difference.

## 3. Results

### 3.1. Reducing property of the modified rice

The reducing power of the samples analyzed by the FRAP assay was evaluated by calculating the amount of  $\text{Fe}^{+2}$  produced by the test samples using the calibration curved of  $\text{FeSO}_4$  (15). The modified rice of JM, NKK, and SH exhibited reducing power as a dose dependent manner as seen in Figure 1. Among the test concentrations, the highest reducing activity was found at the rice concentration of 0.9%. Among the modified rice samples from three rice varieties, SH showed the highest reducing capacity of 122.1  $\mu\text{mol Fe}^{2+}$ /g sample whereas

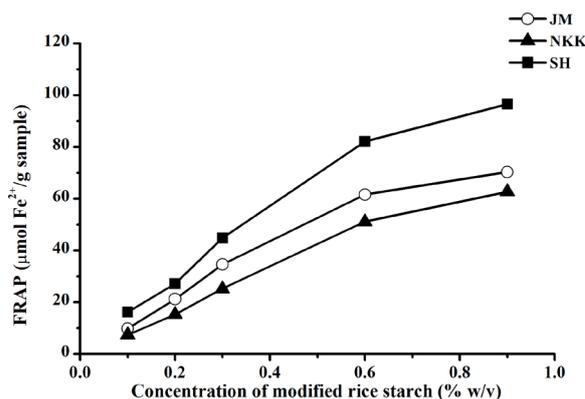


Figure 1. Reducing property of modified rice starch.

JM and NKK showed that of 78.3 and 72.5  $\mu\text{mol Fe}^{2+}$ /g sample, respectively.

### 3.2. Effects of reactant concentration

The reactants in the chemical reaction of AgNPs preparation were the modified rice and  $\text{AgNO}_3$  which both of them were clear and colorless solutions. However, it was noticed that the colorless solutions of the mixtures containing the modified rice and  $\text{AgNO}_3$  changed to yellow color when the AgNPs were formed. After subjecting to UV-Vis spectroscopy, JM-AgNPs, NKK-AgNPs, and SH-AgNPs showed the maximum absorption at 410, 408, and 409 nm, respectively. We used these maximum wavelengths of AgNPs from each rice to determine the amount of the AgNPs obtained from each reactant concentration. The results shown in Figure 2 indicated that change of concentration of  $\text{AgNO}_3$  significantly affected the obtained AgNPs. The higher the concentration of  $\text{AgNO}_3$ , the higher quantity of absorption obtained. The concentration of JM showed significant effect only when 5 mM  $\text{AgNO}_3$  was used whereas that of NKK show no significant difference. Interestingly, the concentration of SH showed significant effect on the obtained AgNPs with all concentrations of  $\text{AgNO}_3$ . Among the test concentrations, the result demonstrated that the highest absorbance of rice-AgNPs was obtained from SH modified rice at 0.9% w/v with  $\text{AgNO}_3$  at 10 mM.

### 3.3. Effects of reaction period

To investigate the role of reaction period on the formation of AgNPs, the gradual generation of AgNPs was monitored by visualization and UV-Vis spectroscopy. It was found that the reaction period affected the yield of AgNPs. During the synthesis process, the color of the mixture changed from colorless to light yellow indicating the beginning of AgNPs production. It was observed that the modified rice of different variety showed different manner of color changing. For example, at the reaction

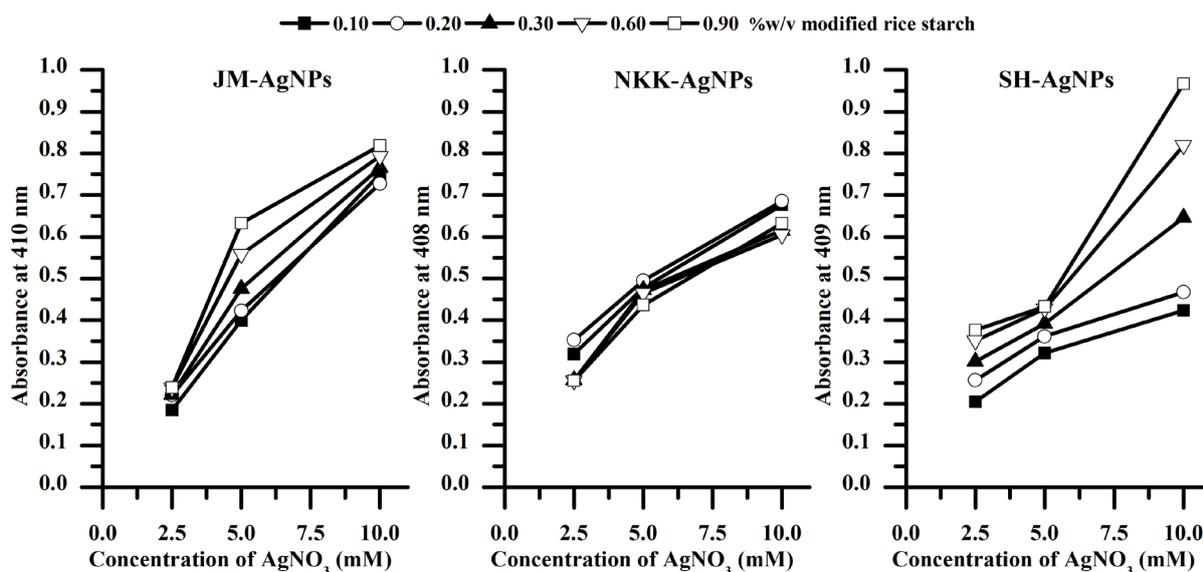


Figure 2. Absorbance of the rice-AgNPs using different concentrations of modified JM, NKK, and SH and AgNO<sub>3</sub> investigated at wavelengths 410, 408, and 409 nm, respectively.

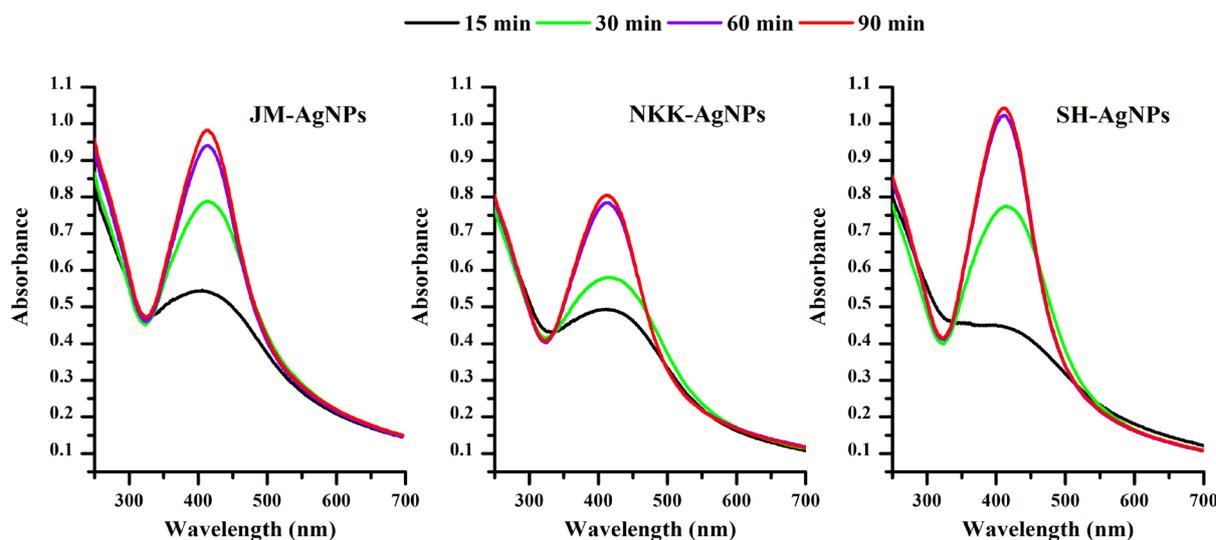


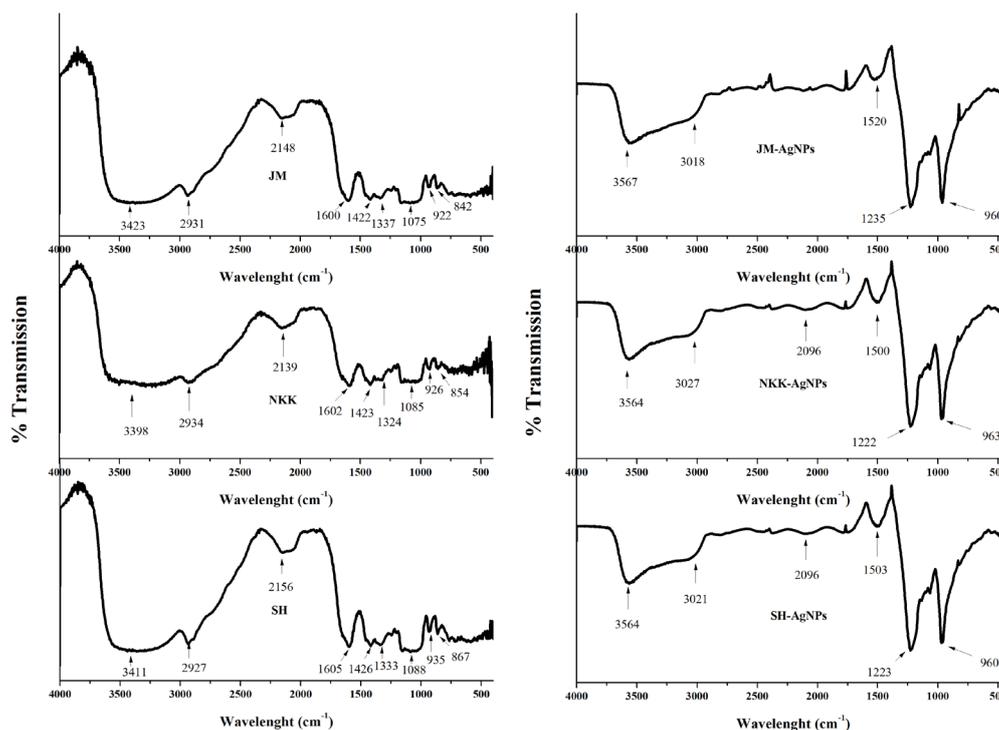
Figure 3. UV-Vis spectra of JM-AgNPs, NKK-AgNPs, and SH-AgNPs synthesized using modified rice starch concentration 0.3% w/v in different reaction periods of 15, 30, 60, and 90 min.

period of 15 min, the color of the mixtures using JM changed rapidly whereas the color of those using SH and NKK gradually changed. At 90 min, it was found that the yellow color of the mixtures using SH was the most intense compared to those using JM and NKK. The obtained JM-AgNPs, NKK-AgNPs, and SH-AgNPs were confirmed by UV-Vis spectrophotometer at the scanning range of 200-700 nm and the quantity of their absorption was observed at their maximum absorption at 410, 408, and 409 nm, respectively. The UV-Vis absorption spectra of the colloidal dispersions of the rice-AgNPs prepared by using JM, NKK, and SH in different reaction period of 15, 30, 60, and 90 min are shown in Figure 3. It was found that with an increase of reaction time, a narrow Plasmon absorption peak around 400 nm remarkably increased due to the AgNPs formation.

It was also noted that the absorption intensity at the reaction periods of 60 and 90 min showed no significant difference. Therefore, the optimal reaction period required for synthesized AgNPs from the modified rice was considered to be 60 min.

#### 3.4. PCS

Size determined by PCS is the hydrodynamic size. The size of rice-AgNPs prepared by using 0.3% modified rice and 10 mM AgNO<sub>3</sub> at a reaction time of 60 min was compared. It was found that the size of AgNPs obtained from different rice variety was different. The size of SH-AgNPs was the smallest of  $80.4 \pm 2.8$  nm whereas the size of JM-AgNPs and NKK-AgNPs was  $113.6 \pm 1.2$  and  $179.0 \pm 5.6$ , respectively. The size distribution was also



**Figure 4.** FTIR spectra of the pure modified rice (left) and the rice-AgNPs (right) showing characteristic peaks at 4,000-400  $\text{cm}^{-1}$ .

different. It was found that the PDI values of JM-AgNPs, NKK-AgNPs, and SH-AgNPs were acceptable range of  $0.214 \pm 0.1$ ,  $0.248 \pm 0.1$ ,  $0.184 \pm 0.1$ , respectively. It was noted that the size distribution of SH-AgNPs was the narrowest. In addition, zeta potential values of these AgNPs were  $-36.3 \pm 0.7$ ,  $-36.1 \pm 1.7$ , and  $-45.9 \pm 1.4$  mV, respectively. It was also noted that the zeta potential of the SH-AgNPs was the highest.

### 3.5. FTIR

The FTIR analysis was carried out to investigate whether the functional group of each modified rice was responsible for synthesis and stabilization of AgNPs. The results showed different stretches of bonds at different peak and the spectra are shown in Figure 4. FTIR spectrum of pure modified rice powder exhibited the typical absorption bands at 3,400, 2,930, 2,140, 1,600, 1,420, 1,337, 1,085, 930, and 850  $\text{cm}^{-1}$ . After reacting with  $\text{AgNO}_3$ , the FTIR spectra of all synthesized rice-AgNPs showed the absorption bands at around 3,560, 3,020, 2,096, 1,500, 1,220, and 960  $\text{cm}^{-1}$ .

### 3.6. Antibacterial activity

The rice-AgNPs was investigated for antibacterial activity against *S. aureus* and *E. coli*. The results showed that AgNPs from all rice varieties had inhibitory effects against both strains as shown in Table 1 and 2, respectively whereas the pure modified rice solution could not inhibit the tested strains. It was found that the

rice-AgNPs obtained from 10 mM  $\text{AgNO}_3$  and 0.3% of modified rice possessed the highest antibacterial activity. The MIC values of JM-AgNPs, NKK-AgNPs, and SH-AgNPs against *S. aureus* were 0.05, 0.1, and 0.025 mg/mL, respectively and against *E. coli* were 0.05, 0.05, and 0.025 mg/mL, respectively. The MBC value of rice-AgNPs from all three rice varieties against *S. aureus* was 0.1 mg/mL whereas that values of JM-AgNPs, NKK-AgNPs, and SH-AgNPs against *E. coli* were 0.1, 0.1, and 0.05 mg/mL, respectively as shown in Table 3.

## 4. Discussion

Rice is composed mainly of starch (> 85%) and other components (< 15%) which are protein, fat, and fiber. Rice starch comprises mainly two types of glucose polymers, amylose, and amylopectin. Okonogi *et al.* have investigated the amylose content in different rice varieties and reported that the amylose content of the non-glutinous SH was found to be the highest of 21% whereas that of the other rice varieties was significantly lower (11). They also reported that the raw rice grains from various rice varieties possess antioxidant activity (10). Moreover, some other research groups reported the difference in phenolic content in different rice varieties (16,17) confirming the antioxidant activity of rice. In the present study, we demonstrated that the rice grains after subjecting to chemical modification still possess antioxidant and reducing activity but in different levels. The reducing activity of the modified rice of non-glutinous rice (JM and SH) was found to be higher than

**Table 1. Inhibition zone of the rice-AgNPs against *S. aureus***

AgNPs	AgNO <sub>3</sub> (mM)	Inhibition zone (mm) Concentration of modified rice (%w/v)				
		0.1	0.2	0.3	0.6	0.9
JM	2.5	NZ	NZ	NZ	NZ	NZ
	5	7.4 ± 0.1	7.4 ± 0.4	7.6 ± 0.3	7.5 ± 0.2	7.2 ± 0.2
	10	7.8 ± 0.2	8.0 ± 0.2	10.6 ± 0.3	9.5 ± 0.5	8.4 ± 0.3
NKK	2.5	NZ	NZ	NZ	NZ	NZ
	5	7.2 ± 0.3	7.1 ± 0.4	7.4 ± 0.4	7.8 ± 0.2	NZ
	10	7.6 ± 0.2	7.8 ± 0.2	8.1 ± 0.6	NZ	NZ
SH	2.5	NZ	7.1 ± 0.3	7.3 ± 0.3	7.6 ± 0.2	7.8 ± 0.2
	5	7.4 ± 0.1	7.6 ± 0.2	7.8 ± 0.2	8.1 ± 0.1	8.0 ± 0.4
	10	8.2 ± 0.2	10.4 ± 0.2	14.2 ± 0.4	11.6 ± 0.2	8.1 ± 0.3

NZ: no inhibition zone. Data were represented as mean ± SD.

**Table 2. Inhibition zone of the rice-AgNPs against *E. coli***

AgNPs	AgNO <sub>3</sub> (mM)	Inhibition zone (mm) Concentration of modified rice (%w/v)				
		0.1	0.2	0.3	0.6	0.9
JM	2.5	NZ	NZ	NZ	NZ	NZ
	5	7.6 ± 0.4	8.1 ± 0.4	9.2 ± 0.2	8.8 ± 0.3	7.8 ± 0.3
	10	10.2 ± 0.2	11.8 ± 0.2	12.4 ± 0.3	12.0 ± 0.5	11.5 ± 0.2
NKK	2.5	NZ	NZ	NZ	NZ	NZ
	5	7.6 ± 0.2	7.8 ± 0.5	7.8 ± 0.2	7.3 ± 0.3	7.1 ± 0.3
	10	8.1 ± 0.3	8.6 ± 0.3	9.2 ± 0.5	8.8 ± 0.4	7.2 ± 0.2
SH	2.5	7.3 ± 0.2	7.5 ± 0.4	8.1 ± 0.4	7.8 ± 0.2	7.4 ± 0.6
	5	7.8 ± 0.2	8.2 ± 0.3	10.1 ± 0.2	9.6 ± 0.3	8.4 ± 0.4
	10	12.4 ± 0.4	14.8 ± 0.2	16.2 ± 0.4	14.1 ± 0.5	12.8 ± 0.3

NZ: no inhibition zone. Data were represented as mean ± SD.

**Table 3. MIC and MBC of the rice-AgNPs (mg/mL)**

Rice-AgNPs	<i>S. aureus</i>		<i>E. coli</i>	
	MIC	MBC	MIC	MBC
JM-AgNPs	0.05	0.1	0.05	0.1
NKK-AgNPs	0.1	0.1	0.05	0.1
SH-AgNPs	0.025	0.1	0.025	0.05

the glutinous rice (NKK). This difference in reducing power leads to the different effect on AgNPs synthesis. As the amylose content in the non-glutinous rice was significantly higher than in the glutinous rice and the results from the FRAP assay indicated that the reducing property of the non-glutinous rice was higher than of the glutinous rice, it is considered that the amylose may also play an important role in reducing property of rice besides the other phenolic components in rice grains. These quantity differences of rice compositions mutually influence the characteristics of their respective modified rice (18). The chemical modification of rice starch yielded different degree of substitution values depending on the amylose content and time of reaction (19). Amylose adopts a left-handed helical form in aqueous solution and can entrap hydrophobic molecules in the helix, which acts as a one-dimensional supramolecular host molecule (20). The end reducing group at C6 of the

starch molecules is looked upon as the reducing agent for fabrication of AgNPs (21). In the same time, soluble starch chains act as capping agent due to their hydroxyl groups to prevent aggregation of the synthesized AgNPs (22). The results in the present study suggest that the modified rice of JM, NKK, and SH still have these functions to reduced AgNO<sub>3</sub> and stabilize AgNPs in the fabrication process.

It has been reported that the concentration of reducing agent is an affecting factor in the intensity of the extinction peak of the synthesized AgNPs spectra. Thus, it denotes that increase concentration causes increase absorption peak height (23). It has also been reported that increasing concentration of AgNO<sub>3</sub>, the maximum absorbance of the obtained AgNPs is increase (24). The result of the present study was according to the previous report for the increasing concentration of AgNO<sub>3</sub>. However, for the reducing agent that the modified rice was used, the result was different. Among the three different rice varieties, only modified SH showed significant effect as increase SH concentration caused increase absorption peak height.

The shape of the UV-Vis spectra gives preliminary information about the size and the size distribution of the AgNPs (25). The results of the present study show that the shape of spectra depended on the reaction period and rice variety. The absorption peaks of AgNPs obtained

from non-glutinous rice (JM and SH) are sharper than that obtained from glutinous rice (NKK). The intense band in the 380-400 nm range indicates much smaller colloidal particles than the another wavelength (26). The rice-AgNPs prepared in various reaction periods of 15-90 min showed the absorption peaks around 400 nm indicating that the fabrication parameters used are suitable to give the AgNPs in a small size. This result also implies that the AgNPs prepared by this synthesis method is very stable without aggregation. This can be confirmed by size measurement using PCS. The particle size of the rice-AgNPs was found to be less than 200 nm depended on the type of rice used in the synthesis process. The AgNPs from non-glutinous rice was smaller than that from the glutinous rice. High zeta potential, either positive or negative, is generally required to ensure stability. In general, systems with zeta potential more than  $\pm 30$  mV are considered to be kinetically stable (27). Our results showed that the rice-AgNPs were negative charge with high value of zeta potential of higher than 30 mV particularly SH-AgNPs which supports long term stability of AgNPs. This result implies that SH-AgNPs are very stable without aggregation.

FTIR spectra provide functional groups like OH or C=O interacted to the surface of AgNPs (28). The IR peaks of the obtained AgNPs in the current study indicate that many functional groups are involved. The peak at  $850\text{ cm}^{-1}$  is considered to be the stretching vibration of CC-CHO (29). The peaks at  $960\text{ cm}^{-1}$  is considered to be the stretching vibrations of C-OCH<sub>3</sub>, C-H stretching of alkenes and C-O stretching aromatic side chain of proteins (30). The absorption band at  $1,085\text{ cm}^{-1}$  could be attributed to stretching vibrations of the C-O bond in either group (31). The peak at  $1,420$  and  $2,930\text{ cm}^{-1}$  are ascribed to the -C-H stretch of the alkyl group. The peak at  $1,600\text{ cm}^{-1}$  the characteristic asymmetrical and symmetrical stretching vibrations of the C=O group (29). The broad peak at  $3,400\text{ cm}^{-1}$  are assigned as OH stretching that could possibly emanate from carbohydrates. The IR spectra of the modified rice of all three test varieties suggest that they contain various active molecules rich in hydroxyl group and carboxyl group that are responsible for reduction of the metallic ion. The presence of these groups on the surface of AgNPs leads to the negative charge and high zeta potential causing the increase stability of the obtained AgNPs.

The antibacterial activity of SH-AgNPs is significantly higher than JM-AgNPs and NKK-AgNPs, respectively. It has been reported that the AgNPs are found to accumulate in the bacterial membrane (32,33). The results in the present study indicate that the rice-AgNPs has slightly higher effective to Gram negative bacteria than Gram positive strains. This result is due to certain difference between Gram positive and Gram negative bacteria. Gram-positive and Gram-negative cells differ markedly in their cell walls which Gram-

positive cells is much thicker with higher amount of peptidoglycan in the cell walls than Gram-negative. The thicker cell wall is therefore extensive practical importance in protecting the cell from penetration of silver ions into the cytoplasm (34-37).

It can be concluded that the modified rice can be used as reducing agent in the synthesis of AgNPs. Among three rice varieties, SH has the highest potential on the synthesis followed by JM and NKK, respectively. The reaction period and concentration of AgNO<sub>3</sub> play an important role in the preparation of AgNPs. The increase absorbance intensity of the rice-AgNPs was dependent on amount of modified SH and AgNO<sub>3</sub> as a reducing agent and a precursor, respectively. The size of AgNPs synthesized from non-glutinous modified rice is smaller than from glutinous rice. SH-AgNPs are the most stable with the highest antibacterial activity to both Gram-positive and Gram-negative bacteria.

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