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Synthesis of silver nanoparticles by using rice husk extracts prepared with acid–alkali pretreatment extraction process

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ABSTRACT

Phenolic acids are the major bioactive compounds responsible for the reducing properties of rice husk. This study quantified the phenolic acid profile, total phenolic content (TPC), reducing power, and ferric-reducing antioxidant power (FRAP) of rice husk extracts ameliorated with acid–alkali pretreatment extraction (AAPE) process. The AAPE rice husk extract exhibited higher phenolic content (TPC: 3428 mg gallic acid equivalent (GAE)/100 g husk) and possessed stronger reducing properties (FRAP of 1433 mM $FeSO_4$/g husk) than the rice husk extract with hot water extraction (TPC: 33 mg GAE/100 g husk, FRAP: 12 mM $FeSO_4$/g husk). A green synthesis process for preparing silver nanoparticles (AgNPs diameter approximately 19 nm) with stable dispersion was obtained, and a conceptual model using caffeic acids as protective agents in synthesized AgNPs was conducted. The recycling of rice husk not only reduces the pollution from the use of chemical reducing agents and dispersants but also enhances the value-addition of the husk.

1. Introduction

Rice grain has the third highest global production. Rice husk (hull) is the outermost layer of the paddy grain and is separated from the rice grains during the milling process. Rice husk can be used as an energy source and contains bioactive compounds such as phenolic acids, which facilitate the antioxidant activity of rice and act as an antioxidant defense system during the growth of rice (Loypimai et al., 2016). Butsat and Siriamornpun (2010) found that the rice husk contains 477.6 mg/100 g total phenolic content (TPC), determined using the Folin–Ciocalteu method, and is the highest portion in rice grain. The TPC in bran ranges from 177.6 to 319.8 mg/100 g, depending on the rice color, and *p*-coumaric acid (*p*-Cou) is the dominant phenolic acid (71%) in the husk, followed by ferulic acid (23%), vanillic acid (3%), and syringic acid (1%) (Goufo and Trindade, 2014).

All phenolic acids in rice grains are primarily derived from the phenylpropanoid biosynthetic pathway, which begins with the conversion of phenylalanine to cinnamic acid catalyzed by phenylalanine ammonia lyase and involves the synthesis of many other phenolic acids (Boudet, 1998). Most phenolic acids in the grains exist in the bound form, and approximately 74% of the total phenolic acids present in rice are in the insoluble bound form (Adom and Liu, 2002; Irakli et al., 2018). The most abundant bound phenolic acid in rice bran is ferulic acid, accounting for almost 50%–65% of the total bound phenolic acids (Irakli et al., 2012). To increase the extraction rate of these phenolic acids, which are esterified to the cell wall, hydrolysis with sodium hydroxide is the preferable process for releasing bound phenolic acids (Shao et al., 2014).

Nanoparticles (NPs) have been widely applied commercially and in research. Silver NPs (AgNPs) possess unique properties that enable their use in biosensors and antibacterial and conductive applications. The Folin–Ciocalteu method is commonly known as a popular and useful TPC assay method. However, the Folin–Ciocalteu assay measures a specimen's reducing capacity, but this does not necessarily represent the content of phenolic acids. Della Pelle and Compagnone (2018) have mentioned the developments of new NPs-based tools and strategies for TPC determination and antioxidant capacity (AOC) evaluation in food since Ag^+ can be reduced, by polyphenols, to Ag°. Della Pelle et al. (2018) have developed a simple and rapid, sensitive AgNPs based spectrophotometric method for AOC assay. This assay is based on the ability of natural polyphenols to reduce Ag^+ and stabilize the produced AgNPs at room temperature. According to the effective and reliable reaction between phenolic acid and NPs, phenolic acids in biological resources can also be used as a green synthesis method for preparing AgNPs.

Physical and chemical methods are the major processes used for the industrial production of AgNPs. However, these processes have the disadvantages of high cost, involving dispersants, and potentially using toxic chemicals. The biosynthesis of NPs has been proposed as an alternative because it is cost-effective, environmentally friendly, and

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nontoxic. Similar to chemical methods, the main mechanism of NP biosynthesis involves the reducing property and NP stabilization of the extracts prepared from sorghum bran (Njagi et al., 2010), banana peel, aloe vera, tansy fruit (Chauhan and Upadhyay, 2012), and other biomaterials (Loo et al., 2012). Reenal and Iruthaya (2015) reported a green synthesis process for AgNPs that used the *Oryza sativa* husk extract by hot water extraction (HWE) process. However, the extraction rate for phenolic acids from plants was limited by HWE, because most phenolic acids combined with lignin in esterified form (Irakli et al., 2012; Formica-Oliveira et al., 2017).

Although Della Pelle and Compagnone (2018) has discussed the principle related to the reducing reaction of phenolic acids to reduce Ag^+ to AgNPs and the stabilization of AgNPs, the extraction ability, phenolic acid composition of rice husk extract, and how these phenolic acids stabilize the dispersion of AgNPs in solution should be further studied. Moreover, establishing a high efficiency process to utilize the bioactive compounds in rice husk may enhance the applications of the husk.

Physical, chemical, and photochemical methods, such as reverse micelles and the thermal decomposition of silver compounds, and the radiation-assisted process used for the synthesis of AgNPs may exert adverse effects in the medical application of these AgNPs. Approximately 20% of paddy weight is the husk, which is discarded during the rice husking process and forms a major proportion of agricultural waste in Taiwan. Synthesis of nanostructure materials with rice husk extract is a favorable method of fabricating AgNPs and an eco-friendly approach because it minimizes the use of substances that are hazardous to human health and the environment. Accordingly, the present study investigated the synthesis of AgNPs using rice husk extract as a reduction agent and stabilizer (Khan et al., 2013). In the present study, we synthesized AgNPs by using rice husk extract with acid–alkali pretreatment extraction (AAPE) as the reducing agent and stabilizer. Acid hydrolysis was performed to destroy the lignin structure, and alkali treatment was utilized to saponify and release the bound phenolic acids from lignin in a high-pressure reactor to increase the extraction rate of phenolic acids and synthesis efficiency of AgNPs. A conceptual model indicating the protective role of caffeic acid (CA) in the synthesized AgNPs was also analyzed and elaborated.

2. Materials and methods

2.1. Materials and chemicals

Fresh rice (*O. sativa* japonica, Kaohsiung 145) husk was collected from the fields surrounding Yilan. The rice husk was dried in hot air until the water content reduced to less than 5% at 60 °C. The dried rice husk was then dry milled, and the powder smaller than 60 mesh was collected as rice husk powder (RHP).

Sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), hydrochloric acid (HCl), methanol (CH_3OH), and ether ($(C_2H_5)_2O$) were purchased from Merck (Darmstadt, Germany). Ethyl acetate ($CH_3COOCH_2CH_3$) was purchased from Macron Chemicals (Center Valley, PA, USA). Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), ferric-reducing antioxidant power (FRAP) reagents, 4-hydroxybenzoic acid (4-hyd BA), 3-hydroxybenzoic acid (3-hyd BA), caffeic acid, syringic acid, gallic acid, vanillic acid, ferulic acid, *p*-Cou, tripyridyltriazine (TPTZ), ferrous sulfate ($FeSO_4$), silver nitrate ($AgNO_3$), and reagents for the preparation of buffer solution were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MS, USA). Protocatechuic acid (proto CA) was purchased from Alfa Aesar (Ward Hill, MA, USA). Acetic acid (CH_3COOH), potassium ferricyanide ($K_3[Fe(CN)_6]$), *trichloroacetic acid (TCA*, CCl_3COOH*)*, and ferric chloride ($FeCl_3$) were purchased from J. T. Baker Chemical Co. (Center Valley, PA, USA).

2.2. Extraction of phenolic acids from rice husk

For AAPE, RHP was hydrolyzed with concentrated sulfuric acid at a ratio of 1:8 (w/v). This mixture, with 2.0 M sulfuric acid, was heated for 40 min at 115 °C in a high-pressure reactor (Model 4522M, Parr Instrument Company, Moline, IL, USA). The cooled reaction product was filtered, and the retentate was washed with deionized water to achieve a neutral pH and was dried for 6 h at 50 °C to yield acid-hydrolyzed rice RHP (AHRHP). AHRHP was reacted with the 8.7% sodium hydroxide at a ratio of 1:30 (w/v). This mixture, with 2.0 M sodium hydroxide, was heated for 118 min at 132 °C in a high-pressure reactor. Concentrated hydrochloric acid was used to adjust the pH of the alkali-treated AHRHP solution to 2.0–3.0 to avoid the pK_{a1} of phenolic acids ($\approx$4–5). The solution was extracted using ether/ethyl acetate (1:1, v/v) at 4 °C and ultrasonic-shocked (LEO-150, Leo Ultrasonic Co., Ltd., New Taipei City, Taiwan) for 30 min. The mixture was then centrifuged at 2500 × *g* for 15 min at 4 °C (Model 7780, Kubota Corporation, Japan), and the supernatant was collected. The aforementioned extraction procedure was performed three times. These extracts were concentrated and blow-dried with nitrogen. The dried extracts were dissolved in methanol and used as the AAPE solution.

For HWE, RHP and deionized water (1:1, w/v) were heated for 30 min at 90 °C. The cooled crude extract was centrifuged at 8000 × *g* for 30 min. The filtered supernatant was used as the HWE solution.

2.3. Determination of phenolic acids in rice husk

The TPC was determined using the Folin–Ciocalteu method (Vichapong et al., 2010). Folin–Ciocalteu method also called the gallic acid equivalence method (GAE), is a mixture of phosphomolybdate ($H_3PMo_{12}O_{40}$) and phosphotungstate ($H_3PW_{12}O_{40}$) used for the colorimetric *in vitro* assay of phenolic and polyphenolic antioxidants. Briefly, 0.1 mL of the extract was introduced into test tubes; subsequently, 0.50 mL of Folin–Ciocalteu reagent and 6.00 mL of deionized water were added. After incubation for 2 min, 2 mL of 15% sodium carbonate was added; the test tubes were incubated for 0.5 min, and the volume was adjusted to 10.0 mL with water. After incubation for 2 h, a spectrophotometer (U2001 UV–Vis Spectrophotometer, Hitachi, Japan) was used to measure the absorbance at 755 nm gallic acid was used as the chemical standard for calibration. The TPC was expressed as mg gallic acid equivalent (GAE) per 100 g of rice husk (mg GAE/100 g husk).

High performance liquid chromatography (HPLC) (Gradient Pump LCP 4100.2; ECOM, Czech Republic) was conducted using a C-18 column (4.6 × 250 mm, Mightysil RP-18 GP 250–4.6, 5 μm, Kanto Kagaku, Japan) at 50 °C ± 0.1 °C, with gradient elution at a flow rate of 1 mL/min, and a UV detector (UV-2070 Plus Intelligent UV/VIS Detector, Jasco, UK) was used to determine phenolic acids at 280 nm. The injection volume was 20 μL, with 4% acetic acid (mobile phase A) and methanol (mobile phase B) as the mobile phase. The gradient condition was as follows: the initial 8% mobile phase B was ramped to 25% (0–15 min) and 35% (15–40 min) and then ramped to 8% (40–45 min). The initial 92% mobile phase A was ramped to 75% (0–15 min) and 35% (15–40 min) and then ramped to 92% (40–45 min). Thereafter, 92% mobile phase A was held for 15 min for washing the column. The samples in methanol were concentrated from 5 to 1 mL by blow drying with nitrogen and were then dissolved in deionized water to obtain a 5-mL volume. The solvent and samples were filtered through a 0.22-μm membrane, and ultrasonic degassing was performed for 10 min. The calibration graphs were constructed by plotting the concentration of the phenolic compounds (mg/L) against the ratio of the peak area of each phenolic compound (gallic acid, proto CA, 4-hyd BA, 3-hyd BA, vanillic acid, caffeic acid, syringic acid, *p*-Cou, and ferulic acid) and the internal standard.

2.4. FRAP assay

The FRAP assay measured the ability of reductants to reduce Fe^{+3} to Fe^{+2}. Under acidic conditions, TPTZ and Fe^{+3} formed the light blue Fe^{+3}–$TPTZ_2$ complex. Under acidic conditions, Fe^{3+}–$TPTZ_2$ complex were reduced and formed the light blue ferrous form and could be monitored by measuring the change in absorption at 593 nm. On the basis of the study of Butsat and Siriamornpun (2010), the stock solutions included 300 mM acetate buffer, pH 3.6; 10 mM TPTZ solution in 40 mM HCl; and 20 mM $FeCl_3{\cdot}6H_2O$ solution. The fresh working solution was warmed to 37 °C before use. Rice husk extracts (300 μL) were reacted with 1.7 mL of the FRAP solution. The absorbance of the mixture at 593 nm was measured after 60 min of the reaction. The results were calculated by plotting standard curves prepared with known concentrations of $FeSO_4$ and are expressed as μmol $FeSO_4$/g husk.

FRAP (%) = $[1 - (A_{593\ nm\ \cdot\ sample}/A_{593\ nm\ \cdot\ blank})] \times 100$.

2.5. Reducing power assay

Phosphate buffer was prepared by mixing 37.5 mL of 0.2 M dibasic sodium phosphate with 62.5 mL of monobasic sodium phosphate, and pH was adjusted to 6.6 by using sodium hydroxide. Various concentrations of the rice husk extracts in the corresponding solvents were mixed with 0.25 mL of phosphate buffer and 0.25 mL of 1% potassium ferricyanide. This mixture was incubated in a water bath at 50 °C for 20 min. After cooling, 0.25 mL of 10% TCA was added, and the mixture was centrifuged at 3000 × *g* for 10 min at 4 °C, whenever necessary. The upper layer of the solution (0.25 mL) was mixed with deionized water (0.25 mL) and freshly prepared ferric chloride solution (0.5 mL). The absorbance was measured at 700 nm. Methanol was utilized as the blank experiment and prepared in a similar manner excluding the samples. Ascorbic acid at various concentrations was used as the standard. The increased absorbance of the reaction mixture indicated an increase in reducing power. Reducing power was measured by varying the concentration of the extract and contact time, and calculated according to Jayanthi and Lalitha (2011).

Reducing power (%) = $A_{700\ nm\ \cdot\ sample} - A700\ _{nm\ \cdot\ blank}$

2.6. Synthesis of AgNPs by using rice husk extracts

AgNPs were biosynthesized according to the processes of Shao et al. (2014), with minor modifications. AgNPs were prepared under the following conditions: the AAPE rice husk extract was diluted 10 times and mixed with 0.001 M silver nitrate at a volume ratio of 1:3 and reacted under pH 10 for 60 min at ambient temperature. The bioreduced aqueous component was used to measure the UV–Vis spectra of the solution at 300–700 nm.

2.7. Analysis of crystal structure of AgNP

The crystal structure of the AgNP was evaluated through X-ray diffraction (XRD; Ultima IV, Rigaku Corporation, Tokyo, Japan). AgNP solution was dripped onto the coverslip and dried with a hot plate (Yellow MAG HS 10/HS 7, Yellow Line, Germany) at 55 °C to form an AgNP film. The film was crushed with a mortar to produce the powder sample. XRD was performed and the data were recorded in the 2θ range of 35°–85° by scanning 5°/min at 40 kV and 20 mA.

2.8. Statistical analysis

All statistical analyses were conducted in triplicate at least, and the results are represented as mean and standard deviation. The data were analyzed and the coefficient of determination was calculated using Statistical Product and Service Solutions (SPSS, SPSS Inc., Chicago, IL, USA).

Table 1
Total phenolic contents, FRAP values, and reducing power of rice husk extracts.

Sample	TPC (mg GAE/100 g husk)	FRAP (mM FeSO4/g husk)	Reducing Power (%)
AAPE	3428.2 ± 20.6	1432.7 ± 6.6	98276.2 ± 9672.3
HWE	33.3 ± 3.3	12.1 ± 0.2	27363.7 ± 2375.1

Values are expressed as mean ± SD, n = 3.

3. Results and discussion

3.1. Reducing properties of rice husk extract

The AAPE rice husk extract showed a higher reducing property than the HWE rice husk extract (Table 1). The HWE rice husk extract exhibited a low FRAP because most phenolic acids in rice husk exist in the insoluble bound form (Irakli et al., 2012). Furthermore, approximately 6%, 17%–30%, and 66%–80% of phenolic acids in each type of grain are in the free, soluble bound, and insoluble bound forms, respectively. Such insoluble bound form accounts for almost 50%–65% of the total bound phenolic acids (Li and Lenhart, 2012). The acid treatment disrupted the interaction among lignin, cellulose, and hemicelluloses, which increased the voids in rice husk after the destruction of cellulose crystals, and the rice husk was then swollen after alkali treatment. Saponification occurred between the bound phenolic acids and ester bonds of lignin, leading to the subsequent release of phenolic acids. Therefore, because more phenolic acids were released, the AAPE rice husk extract exhibited a higher FRAP than the HWE rice husk extract.

The phenolic acids-rich rice husk extract might be used as safe reducing agents for AgNP synthesis. Szydlowska-Czerniak et al. (2012) reported that the phenol was converted into a phenate ion and becomes more reducible in the AgNP synthesis by sinapic acid. The sinapic acid was oxidized by Ag^+ and converted to the corresponding quinone structure, and the Ag^+ were reduced to AgNPs at the same time. Vichapong et al. (2010) also reported a strong relationship between TPC and the antioxidation ability of rice. Therefore, TPC determination becomes important while evaluating the reducing properties of rice husk extract. The TPC and FRAP of the AAPE rice husk extract (mg GAE/100 g husk) were almost 100 times higher than the HWE rice husk extract (Table 1). Kim et al. (2011) also found that a higher TPC is associated with a higher reducing property of rice husk, and they demonstrated that phenolic acids contribute to the reducing properties of rice husk. However, the disparity in reducing power between the AAPE and HWE rice husk extracts was not as large as that in FRAP.

3.2. Phenolic acid composition of rice husk extract

To determine the phenolic acid composition of rice husk, nine types of phenolic acids were analyzed through HPLC (Fig. 1). Because more than 75% of the mobile phase was deionized water, and the AAPE sample was dissolved in methanol, the fronting peaks were observed (Fig. 1a) if the AAPE sample was directly injected into the HPLC column. This phenomenon often occurs when the viscosity, pH, or polarity of the sample are incompatible with the mobile phase and is known as sample diluent effects (Ruta et al., 2010). The sample diluent effects also cause variations in the residence time of the sample (Ruta et al., 2010). These effects account for the position of phenolic acid peaks in Fig. 1a being different from that in Fig. 1b. Therefore, the AAPE rice husk extract was concentrated and dissolved in deionized water to prevent this phenomenon from affecting the interpretation of HPLC chromatograms.

p-Cou is the main phenolic acid in rice husk, and its peak was observed at the residence time of 25 min in Fig. 1b. By contrast, ferulic

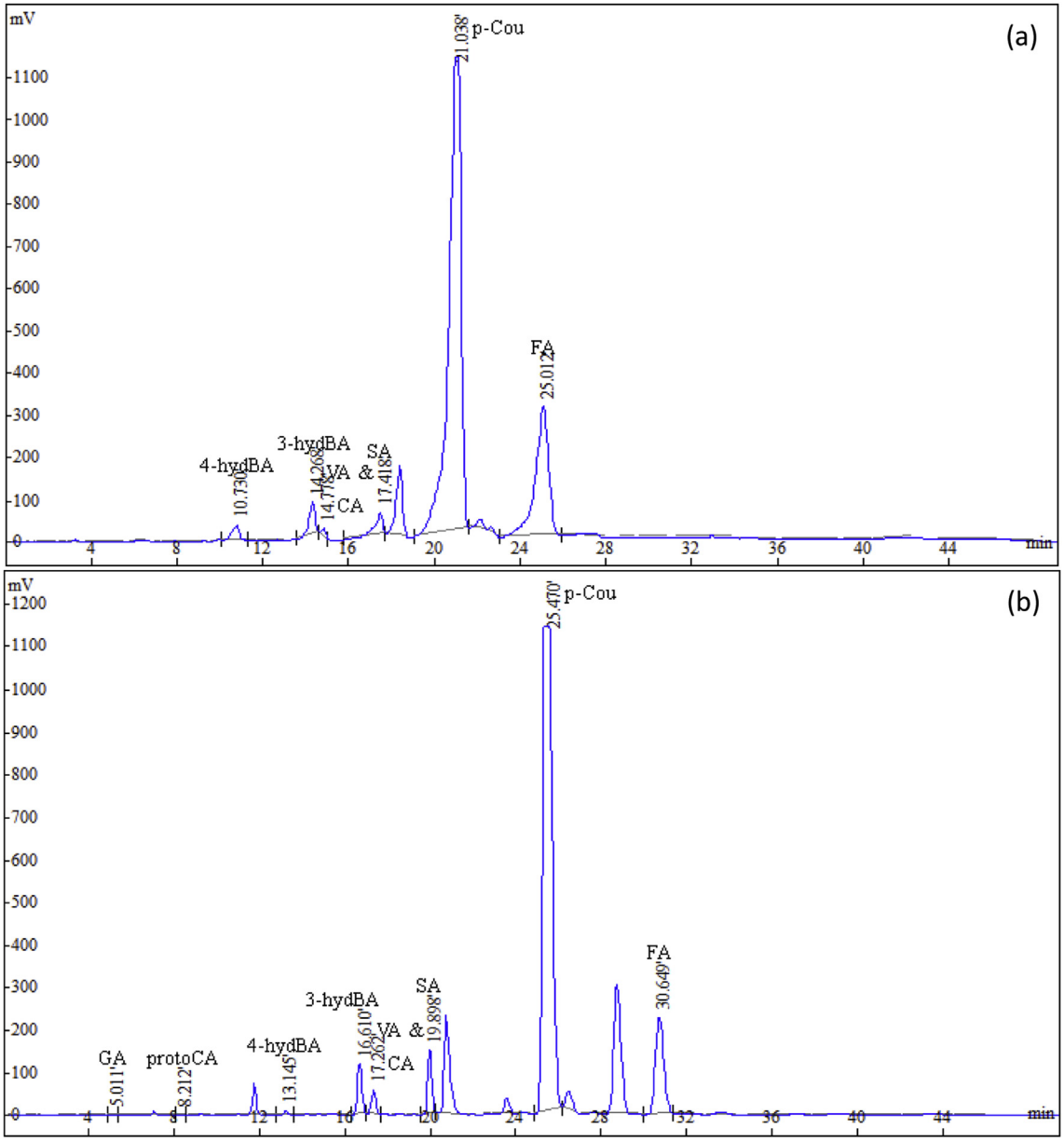

Fig. 1. HPLC chromatograms of phenolic acids in the AAPE rice husk extract: direct injection of the sample (a) and the concentrated sample dissolved in water and injected (b). Peaks: (GA)–gallic acid, (proto CA)–protocatechuic acid, (4-hyd BA)–4-hydroxybenzoic acid, (3-hyd BA)–3-hydroxybenzoic acid, (VA)–vanillic acid, (CA)–caffeic acid, (SA)–syringic acid, (*p*-Cou)–p-coumaric acid, and (FA)–ferulic acid.

acid is the main phenolic acid in bran, germ, or endosperm of rice (Butsat and Siriamornpun, 2010; Goufo and Trindade, 2014; Shao et al., 2014), and the composition ratio of ferulic acid in free: soluble bound form: insoluble bound form is approximately 0.1:1:100 (Adom and Liu, 2002). This observation is because ferulic acid tends to bind tightly to arabinoxylan in aleurone cell walls and parts of bran in the ester form. The distribution of ferulic acid is more uniform throughout the whole grain; in contrast to ferulic acid, *p*-Cou and vanillic acid tend to bind to lignin in the cell wall (Zhou et al., 2004). Moreover, *p*-Cou is the precursor of lignin (Hisano et al., 2009), and rice husk possesses a high lignin content (15%–22%) (Patel et al., 1987). Thus, in this study, the highest *p*-Cou content was obtained in the AAPE rice husk extract.

To improve the low extraction rate from rice husk by HWE, acid hydrolysis was performed to destroy the lignin structure, and alkali treatment was utilized to saponify and release the bound phenolic acids from lignin by AAPE. Table 2 lists the phenolic acid composition of the rice husk extract. The AAPE rice husk extract contained 762.53 ppm of

Table 2
Phenolic acid contents in rice husk extracts prepared from HWE and AAPE methods.

Phenolic acid	Amount in HWE (μg/mL)	Amount in AAPE (μg/mL)
Gallic acid	2.23 ± 0.13	0.50 ± 0.14
Protocatechuic acid	1.16 ± 0.09	0.90 ± 0.24
4-hydroxybenzoic acid	1.17 ± 0.09	5.38 ± 0.13
3-hydroxybenzoic acid	16.93 ± 1.50	170.27 ± 4.65
Vanillic acid & caffeic acid	3.43 ± 0.95	22.26 ± 5.33
Syringic acid	0.83 ± 0.08	31.84 ± 6.18
p-Coumaric acid	25.29 ± 1.07	432.84 ± 3.41
Ferulic acid	2.72 ± 0.06	98.54 ± 0.69
Total	53.76	762.53

Values are expressed as mean ± SD, n = 3.

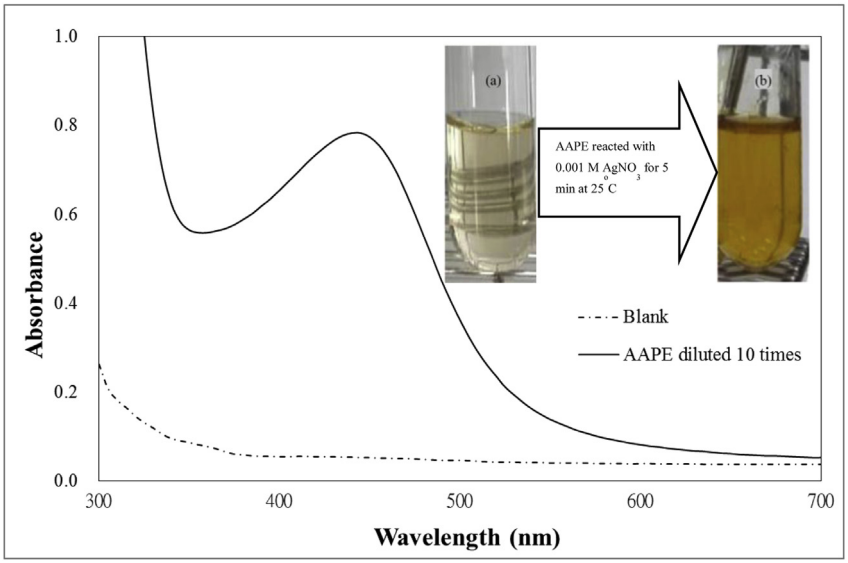

Fig. 2. UV–Vis spectrum of synthesized AgNPs by using 1 mL of AAPE diluted 10 times, adjusted to pH 10, and reacted with 3 mL of 0.001 M AgNO3 at 25 °C after 60 min.

phenolic acids, which is much higher than the HWE rice husk extract. Among the phenolic acids, only gallic acid and proto CA, the contents of which were very low, were less in the AAPE rice husk extract than in the HWE rice husk extract. It is speculated that these two phenolic acids possess higher polarity and are oxidized easily under high temperature, acid, and alkali environments.

3.3. Synthesis of AgNPs by using rice husk extract

According to the HPLC results, the rice husk extract possesses abundant phenolic acid content, and 1525 mg phenolic acids were obtained from 100 g rice husk through AAPE. The rice husk extracts were pale white before the addition of silver ions and changed to yellowish brown after 5 min of incubation with silver ions (inset, Fig. 2), indicating the formation of AgNPs, because AgNP-containing solution appears yellowish brown (Agnihotri et al., 2014).

Özyürek et al. (2012) found that the gallic acid added AgNPs solution exhibited a band at 423 nm which was due to surface plasmon resonance of AgNPs and it may be attributed to the collective oscillation of electron gas in the particles with a periodic change in the electronic density at the surface. Such generation of polyphenol mediated-AgNPs proceeds with further reduction of Ag^+ to Ag by the polyphenolics (e.g., gallic acid) on the citrate-stabilized AgNP seeds. Therefore, UV–Vis spectroscopy is an important technique for analyzing and identifying the formation of silver–metal NPs in aqueous solution (Kotakadi et al., 2013). In this study, an SPR spectrum of AgNPs was obtained at approximately 450 nm, with the AAPE rice husk extract changing to light yellow brown after a 60-min reaction with silver nitrite (Fig. 2). According to Stamplecoskie and Scaiano (2010), peaks at approximately 450 nm correspond to the presence of AgNP spheres in the sample. A similar UV–Vis spectrum was observed for the synthesis of AgNPs from the extracts of *Macrotyloma uniflorum* seeds (Vidhu et al., 2011), *Ananascomosus* L. (Ahmad and Sharma, 2012), and *Camellia sinensis* var. *assamica* (Loo et al., 2012).

The size, shape, and crystal morphology of the AgNP synthesized using rice husk were further characterized through XRD (Fig. 3). The black and gray lines in Fig. 4 represent the spectra of the biosynthesized AgNPs and the standard Ag, respectively. Five main characteristic diffraction peaks were observed in both spectra at 2θ = 38, 44, 64, 77, and 81, which correspond to the (111), (200), (220), (311), and (222) crystallographic planes of face-centered cubic Ag crystals, respectively (Vidhu et al., 2011). No peaks from any other phase were observed, revealing that single-phase, cubic-structure AgNPs were obtained directly.

The crystallite size of the AgNPs synthesized using AAPE rice husk extract was found to be less than 20 nm (Fig. 3b), which is similar to the size of AgNPs biosynthesized using the *T. vulgare* fruit (16 nm; Dubey et al., 2010), *Macrotyloma uniflorum* seed (17 nm; Vidhu et al., 2011), and *O. sanctum* leaves (18 nm; Ramteke et al., 2012) estimated by XRD.

3.4. Conceptual model of AgNP biosynthesized with rice husk extract

Phytochemicals are considered the major bioactive components in the biosynthesis of AgNPs (Vidhu et al., 2011; Khan et al., 2013), and phenolic acids are the major phytochemicals in rice husk. Phenolic acids have been reported to possess hydroxyl and carbonyl groups that can bind to metals. Phenolic compounds may inactivate ions through chelation. This chelating ability of phenolic compounds is probably related to the high nucleophilic character of the aromatic rings (Vidhu et al., 2011). Therefore, phenolic acids are expected to have a high antioxidant activity. According to the Fourier transform infrared results of Khan et al. (2013), the peaks in the plant extract spectrum represented that AgNPs were bound to oxygen from the hydroxyl groups in *Pulicaria glutinosa* compounds based on the formation of a new C = O group that was an aldehyde, a ketone, or a carboxylic acid. This finding strongly suggests that Ag was reduced by some hydroxyl groups that were oxidized at the expense of Ag because of Ag^+ reduction.

The AgNPs in aqueous solution that were prepared from synthesis by using the AAPE rice husk extract were dispersed evenly and stably because no color change and sedimentation were observed during their storage for 5 months at ambient temperature. These AgNPs can be inferred to be surrounded by phenolic acids that formed negatively charged layers and presented a space hindrance to prevent the aggregation of AgNPs by electrostatic repulsion. Fig. 4 displays a conceptual model indicating that caffeic acid is a protective agent in the synthesized AgNPs. Caffeic acid belongs to o-diphenols, assess the best potential to be higher reducing power because of its o-diphenolic structures (Del Carlo et al., 2012; Della Pelle et al., 2017). The 3,4 position of dihydroxylation on the phenolic ring in caffeic acid contributes to its high antioxidant activity. Caffeic acid is expected to have a higher antioxidant activity because of additional conjugation in the propanoic side chain, which might facilitate electron delocalization by resonance between the aromatic ring and propanoic group (Vidhu et al., 2011). The reduced AgNPs were also surrounded by a large number of

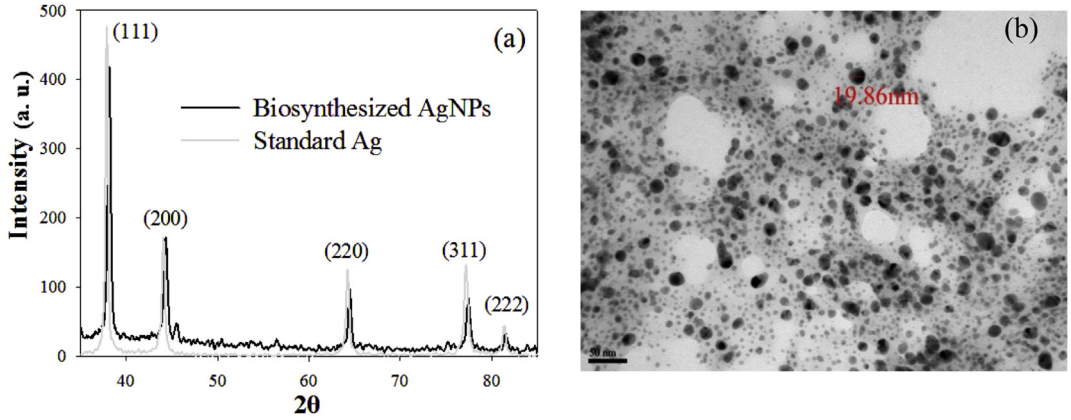

Fig. 3. (a) XRD pattern (JCPDS silver file No. 04–0783) and (b) TEM image (300,000×) of synthesized AgNPs by using 1 mL of AAPE diluted 10 times, adjusted to pH 10, and reacted with 3 mL of 0.001 M AgNO3 at 25 °C after 60 min.

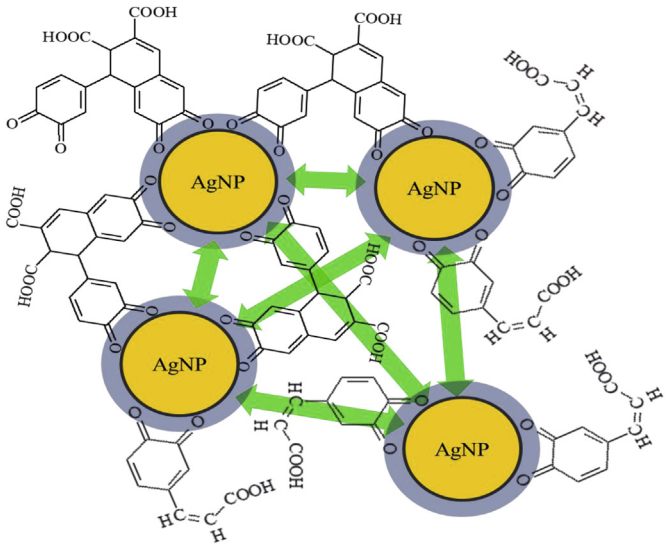

Fig. 4. A conceptual model of CA as a protective agent in the synthesized AgNPs.

C = O groups on quinone compounds derived from caffeic acid and were immobilized, resulting in the stable dispersion of AgNPs in solution.

4. Conclusions

AAPE substantially increased phenolic acid extraction from the rice husk compared with conventional HWE. AgNPs dispersed evenly and stably in aqueous solution can be successfully biosynthesized by the reduction of the rice husk extract through AAPE. The abundant phenolic acids in the AAPE rice extract were considered not only as reducing agents but also as protective agents that enabled the stable dispersion of green synthesized AgNPs. Ag was reduced by the hydroxyl groups of phenolic acids that were oxidized, and the reduced AgNPs were surrounded by a large number of C = O groups, thus facilitating the stable dispersion of AgNPs in aqueous solution. This green synthesis process reduces the use of chemical reducing agents and dispersants. Moreover, rice husk is the main byproduct of rice production. Thus, the recycling of rice husk not only reduces pollution but also enhances the applications of the husk.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jcs.2018.06.002.

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