Original Article

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Effect of rice variety and modification on antioxidant and anti-inflammatory activities

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Summary **The effects of variety and modification of rice on its antioxidant and anti-inflammatory activities were investigated. White rice varieties; Jasmine (JM) and Saohai (SH), and pigmented rice varieties; Doisket (DS) and Homnil (HN) were used. The modified rice samples were obtained from chemical modification using etherification reaction. The activities of the modified rice samples were compared with the ethanol extracts of the raw rice at the same rice concentration. Antioxidant activity was measured by the free radical scavenging activity tests and ferric reducing power assay. Results indicated that the ethanol extracts of raw rice had higher antioxidant activity than the modified rice. Among the raw rice tested, the pigmented rice showed higher antioxidant activity than white rice. Trolox equivalent antioxidant capacity values from free radical scavenging activity test were revealed that 50% ethanol extracts of HN and DS possessed the highest antioxidant activity. Ferric reducing power assay showed that 50% ethanol extracts of DS had the highest antioxidant activity. The anti-inflammatory activity was evaluated *in vitro* using a lipopolysaccharide-stimulated RAW264.7 macrophage cell model with enzyme-linked immunosorbent assay. Absolute ethanol extracts of HN reduced interleukin-6 secretion whereas that of DS suppressed interleukin-6 and tumor necrosis factor -α secretion. These results indicate that variety of rice, chemical modification, and extracting solvent were the factors that play an important role on antioxidant and anti-inflammatory activity. This study supports the potential use of the pigmented rice, especially DS, as a promising choice of a natural source because of its antioxidant and anti-inflammatory activities.**

Keywords: White rice, pigmented rice, modified rice, antioxidant, anti-inflammatory

1. Introduction

Oxidative stress is the resulting from the accumulation of reactive oxygen species and it can induce inflammatory cells to produce inflammatory mediators, such as cytokines and chemokines (*1*). Pro-inflammatory cytokines, such as tumor necrosis factor (TNF) -α, interferon-γ, interleukin (IL) -1β, IL-6, and IL-18, play an important role in signal transduction cascades during the progression of inflammation (*2*). Oxidative stress and inflammation increase the risk of various chronic diseases such as heart disease, cancer, diabetes (*3*), and Alzheimer's disease (*4*). There is a need for alternative treatment such as natural remedies due to conventional treatments show several side effects. Moreover, several studies have demonstrated the importance of diet in the control of chronic diseases. It has been reported that fruits, legumes, and vegetables, as well as grains consumption (*5*), have been associated with reduced the risk of chronic disease development (*6*). This could be attributed to the presence of natural bioactive compounds in these foods (*7*).

Rice (*Oryza sativa* L.) is one of the most important cereal grains and the economic agriculture in Asia. It

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is the principal staple food for more than half of the world's population. Based on the color of rice grains, rice can be categorized into two groups, non-pigmented rice and pigmented rice. Non-pigmented or white rice grains are white or pale yellow whereas pigmented rice grains show specific color, such as red, purple, and black. Rice grain is a rich source of many bioactive compounds including phenolics, flavonoids, and sterol derivatives (*8*). Phenolic compounds such as ferulic acid, p-coumaric and diferulate are presented in rice grains (*9*). Those compounds directly scavenge some reactive species as chain breaking antioxidants and suppress lipid peroxidation recycling other antioxidants, such as tocopherol (*10*). Some phenolic compounds may bind pro-oxidant metals, such as iron and copper, preventing the formation of free radicals from these pro-oxidants while simultaneously maintaining their capacity to scavenge free radicals. Previous studies indicated that phenolics might also suppress gene expression for pro-inflammatory factors (*11*).

Recent studies have reported that rice exhibit the potential to reduce risk of disease due to their antioxidant and anti-inflammatory activities. Especially in the pigmented rice, it has been reported that the pigmented rice contains natural anthocyanin compounds, such as cyanidin 3-glucoside and peonidin 3-glucoside (*12*), which possess antioxidant and anti-inflammatory activities (*13*). Those compounds have the potential to reduce the risk of chronic disease (*14*) and help to prevent cellular damage from oxidative stress.

Modification of rice starch has been shown to access various valuable features to the rice. Modified rice is widely used in pharmaceutical field because of their less toxicity and biodegradable properties. It can be used as gelling agents (*15,16*), flocculants, thickeners, stabilizers, fillers, binders, and disintegrants (*17*). Our previous studies reported that the modified rice obtained from chemical modification by etherification method could improve some property of rice (*15*) and it can be feasible to be useful in pharmaceutical preparation (*16,18*).

Although the antioxidant and anti-inflammatory activities of rice have been previously investigated (*19,20*), however, there is limited information available about local rice varieties that were chosen for these studies. Moreover, less investigation on the effect of chemical modification method to antioxidant and anti-inflammatory activity of modified rice was found. The aim of the present study is to investigate the effects of rice variety and rice modification on their antioxidant and anti-inflammatory properties.

2. Materials and Methods

2.1. *Materials*

Thai white rice grain varieties; Jasmine (JM) and Saohai (SH), and Thai pigmented rice grain varieties; Doisket (DS) and Homnil (HN), were obtained from a different rice cultivation area of Chiang Mai province, Thailand. Monochloroacetic acid, silver nitrate, Trolox, potassium persulfate, 2,'-azinobis-(3-ethylbenzothiazoline-6-sulfonicacid) diammonium salt (ABTS), 2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-S-triazine (TPTZ), thiazolyl blue tetrazolium bromide (MTT), sodium dodecyl sulfate (SDS), hydrochloric acid (HCl), and lipopolysaccharide (LPS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's minimum essential medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin, and L-glutamine were obtained from Life Technologies (Carlsbad, CA, USA). Macrophage RAW264.7 cells were purchased from an American type culture collection. Enzyme linked immunosorbent assay (ELISA) kits were purchased from eBioscience (San Diego, CA, USA). Methanol, ethanol and glacial acetic acid were from RCI Lab-scan Co., Ltd. (Bangkok, Thailand). All other chemicals and solvents were of analytical grade or the highest grade available.

2.2. *Sample preparation*

2.2.1. *Preparation of raw rice and rice extracts*

Raw rice powder was prepared by wet milling method (*21*). Each rice sample was accurately weighed and extracted with absolute ethanol (Abs-EtOH) and 50% EtOH (50-EtOH), at a ratio of rice powder to a solvent of 1:10 w/v. The mixtures were stirred at 750 rpm for 24 h at room temperature. The mixtures were filtered through Whatman No. 1 filter paper. The filtrate was subjected to a rotary evaporator (EYELA N-1000, NY, USA) to remove the solvent. The obtained extracts of Abs-EtOH from JM (JM-Abs-EtOH), SH (SH-Abs-EtOH), DS (DS-Abs-EtOH), and HN (HN-Abs-EtOH) and those of 50-EtOH of JM (JM-50-EtOH), SH (SH-50-EtOH), DS (DS-50-EtOH), and HN (HN-50-EtOH) were kept at 4°C for further studies.

2.2.2. *Preparation of modified rice.*

Raw rice powder was modified using etherification method previously described by Okonogi *et al*. (*15*) with some modification. Briefly, 50% (w/w) sodium hydroxide aqueous solution was mixed with methanol at a ratio of 1:4 (w/w) in a 100-mL three necked round-bottom flask. The raw rice powder was added and stirred at room temperature until homogenous slurry was obtained. After that, proper amount of monochloroacetic acid was added. The temperature of the mixture was controlled at 50 ± 1°C for 3 h with continuous stirring. At the end of the reaction, the mixture was neutralized to pH 7.0. The solid phase was collected and washed by 80% (w/w) methanol until the filtrate testing for chloride by using silver nitrate test

was negative. The obtained slurry was dried at 50°C for 48 h. The obtained dried solid was pulverized and passed through the 80-mesh sieve. After passing the sieve, the obtained modified rice samples of JM (JM-M), SH (SH-M), DS (DS-M) and HN (HN-M) were kept in a desiccator for further use.

2.3. *Sample preparation for antioxidant and anti-inflammatory activity tests*

The samples used in antioxidant and anti-inflammatory activity tests were prepared as following. Exact amount of 4 g of raw rice powder was extracted using Abs-EtOH and 50-EtOH as extracting solvent. The solvents of the extract solution were evaporated. The yield of the extracts was recorded. The obtained semisolid mass extracts from Abs-EtOH and 50-EtOH were diluted with Abs-EtOH and 50-EtOH, respectively, to obtain the clear sample solutions of 40 mL. Meanwhile, exact amount of 4 g of modified rice was used without extraction. The modified rice powder samples were dissolved in water to obtain the clear sample solutions and the volume was adjusted with water to 40 mL. The sample solutions of rice extracts and modified rice solutions were further diluted with their respectively vehicles, *e.g*. Abs-EtOH and 50-EtOH for the rice extracts and water for the modified rice to obtain the 1,000-fold dilutions. This dilution of each sample was used for determination of antioxidant activity and anti-inflammatory activity test.

2.4. *Determination of antioxidant activity*

2.4.1. *Free-radical scavenging activity on ABTS*

ABTS assay described previously by Saeio *et al*. (*22*) was used in this experiment. Briefly, free radical ABTS was generated by reacting ABTS solution with potassium persulfate. The mixture was prevented from light and left to stand at room temperature for 12 h. Then, the mixture was diluted with Abs-EtOH to obtain the absorbance of approximately 0.7 units at 750 nm. The exact amount of 20 µL solution of each rice dilution was mixed with 180 µL ABTS free radical solution. The mixture was left to stand for 5 min at room temperature then the absorbance at 750 nm was recorded using microplate reader (Biorad 680, Hercules, CA, USA). Trolox was used to construct a standard curve. The antioxidant activity is expressed as Trolox equivalent antioxidant capacity (TEAC) in millimolar concentration of Trolox which antioxidant capacity is equivalent to 1 mg of the test sample.

2.4.2. *Free-radical scavenging activity on DPPH*

DPPH assay described previously by Okonogi *et al*. (*23*) was used in this experiment. Briefly, the solution of DPPH free radicals was prepared by dissolving the free radicals in Abs-EtOH to a concentration of 100 µM. The exact amount of 20 µL solution of each rice dilution was mixed with 180 µL DPPH free radical solution. The mixture was protected from light and left to stand for 30 min at room temperature. The amount of DPPH remaining in each period of stand was determined at 520 nm using the microplate reader. Trolox was used to construct a standard curve. The antioxidant activity is expressed as TEAC in millimolar concentration of Trolox which antioxidant capacity is equivalent to 1 mg of the test sample.

2.4.3. *Ferric reducing antioxidant power (FRAP) assay*

FRAP assay was determined according to a procedure described previously by Tachakittirungrod *et al*. (*24*). Briefly, the FRAP reagent was prepared by mixing TPTZ solution with $FeCl_3$ solution. The exact amount of 20 µL solution of each rice dilution was mixed with 180 µL FRAP reagent. After 5 min of mixing, the absorbance was taken at 595 nm using the microplate reader. The standard curve was constructed using $FeSO_4$ solution. The antioxidant compound will reduce the ferric ion into ferrous ion; the later reacts with TPTZ to form a blue complex which increases the absorption. The reducing power was expressed as equivalent concentration (EC). This parameter was defined as the concentration of antioxidant having a ferric reducing ability equivalent to that of 1 mM $FeSO_4$.

2.5. *Determination of anti-inflammatory activity*

RAW 264.7 macrophages were cultured in DMEM media containing 10% FBS, 4 mM L-glutamine, and 100 units-penicillin-streptomycin. The cells were seeded with a density of 2×10^6 cells per well into a 24 well plate and incubated at 37°C for 24h. Subsequently, the exact amount of 1 µL solution of each rice dilution was added to each well and the plates were incubated at 37°C for 3h. Then, the macrophages were activated by adding 1 µg/mL of LPS and the plate was further incubated at 37°C for 24 h. The positive control was the plates treated with LPS without the solution of rice dilution. The negative control was the plates treated without neither LPS nor the solution of rice dilution. The 500 µL supernatant of each well was removed and kept in −20°C for the ELISA assay.

MTT assay was used to determine cell viability. The 100 µL solution of 5 mg/mL MTT was added into the plates and incubated at 37°C for 2 h. After that, the supernatant was removed and the cells were lysed with 500 µL of lysis buffer (10% SDS in 0.01N HCl).The absorbance was recorded at 570 nm with reference at 690 nm using microplate reader.

Pro-inflammatory cytokines; IL-6 and TNF-α, in the cell supernatants were quantified using ELISA

kit according to the manufacturer's instructions. The absorbance was recorded at 450 nm with a reference at 570 nm using the microplate reader. The variation from cell density was reduced by using MTT values for normalization. The amount of cytokines of the positive control was defined as 100%. The results of the samples were calculated as a percent of this value. The inflammation assay was repeated in triplicate on independent days. The influence of the test compounds on cytokine secretion was defined as significant if the level of the positive control was changed by at least 25%.

2.6. *Statistical analysis*

Descriptive statistics for continuous variables were calculated and reported as a mean ± standard deviation (SD). Data were analyzed using a one-way analysis of variance (ANOVA) and Duncan's multiple range test ($p < 0.05$) using SPSS statistic software version 22.

3. Results

3.1. *Rice samples*

The raw rice powders of white rice grains (JM and SH) and pigmented rice grains (DS and HN) appeared different color. JM powder was white whereas SH powder was yellowish white. The color of both pigmented rice powder was purple. After extraction with Abs-EtOH and 50-EtOH, all rice extracts were clear viscous liquid. The color of white rice extracts was light brown-yellow for SH and light yellow for JM. The color of the pigmented rice extracts obtained from Abs-EtOH was dark purple whereas that obtained from 50-EtOH was different. The color of HN-50-EtOH was dark purple-green whereas that of DS-50-EtOH was dark red-purple.

The yield of all rice extracts ranged from 0.96% to 6.18 % (w/w), as shown in Table 1. Among the rice extracts obtained from Abs-EtOH, DS gave the highest yield of 6.18% whereas JM gave the lowest yield of 0.96%. Among the rice extracts obtained from 50-EtOH, DS also gave the highest yield of 3.91% whereas JM gave the lowest yield of 0.72%.

Table 1. Yield of rice extracts

Samples	Yield (%)*
SH-Abs-EtOH	1.46 ± 0.04
SH-50-EtOH	0.99 ± 0.01
JM-Abs-EtOH	0.96 ± 0.01
JM-50-EtOH	0.72 ± 0.01
HN-Abs-EtOH	2.26 ± 0.03
HN-50-EtOH	3.43 ± 0.02
DS-Abs-EtOH	6.18 ± 0.02
DS-50-EtOH	3.93 ± 0.03

*yield (%) = dry weight of extracts/raw weight of sample × 100%.

After subjecting to the chemical modification, the color of rice powder was changed to off-white. The obtained modified rice powders showed high ability to dissolve in water. Ethanol extraction was also carried out with the obtained modified rice samples using the same manner as the extraction of the raw rice. It was found that no yield of extract was obtained from all modified rice samples.

3.2. *Antioxidant activities of rice samples*

3.2.1. *ABTS radical scavenging activity*

The results from ABTS assay are presented in Figure 1. Raw rice extracts showed higher antioxidant activity than their modified rice ($p < 0.05$). Among the raw rice extracts, the samples extracted by 50-EtOH gave higher activity than those extracted by Abs-EtOH. HN-50-EtOH and DS-50-EtOH possessed the highest free radical scavenging property with TEAC values of 1.18 ± 0.17 mM/mg and 1.07 ± 0.07 mM/mg followed by SH-50-EtOH and JM-50-EtOH, respectively. The rice samples extracted by Abs-EtOH showed lower TEAC values than those extracted by 50-EtOH. Among the rice samples extracted by Abs-EtOH samples, HN-Abs-EtOH exhibited the highest free radical scavenging property with TEAC values of 0.62 ± 0.12 mM/mg followed by DS-Abs-EtOH and JM-Abs-EtOH, respectively. The lowest TEAC was found in SH-Abs-EtOH.

3.2.2. *DPPH radical scavenging activity*

The results of DPPH radical scavenging assay are shown in Figure 2. The raw rice extracts showed higher antioxidant activity than their modified rice ($p < 0.05$). Among the raw rice extracts, DS-50-EtOH and HN-50-EtOH showed the highest scavenging activity with TEAC values of 1.06 ± 0.07 mM/mg and 1.05 ± 0.02

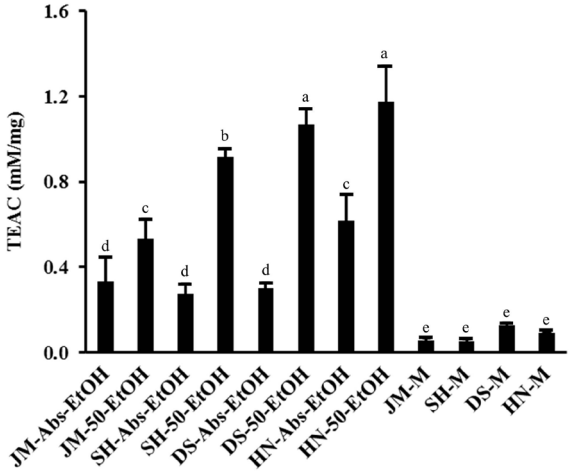

Figure 1. TEAC values of rice samples from ABTS assay. Different letters indicate significant differences ($p < 0.05$).

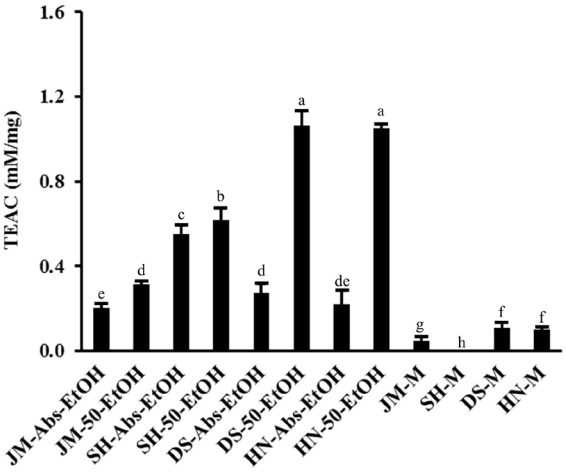

Figure 2. TEAC values of rice samples from DPPH assay. Different letters indicate significant differences ($p < 0.05$).

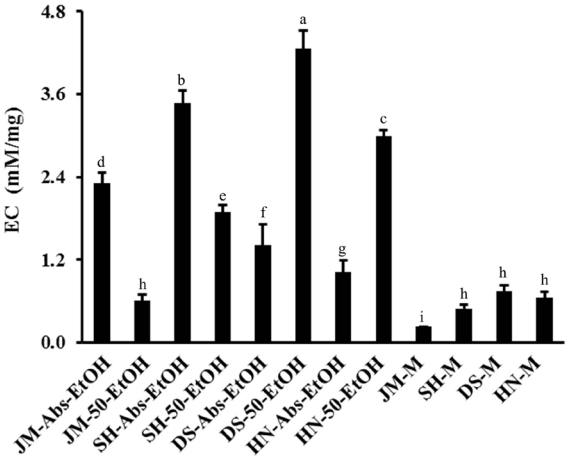

Figure 3. EC values of rice samples. Different letters indicate significant differences ($p < 0.05$).

mM/mg followed by SH-Abs-EtOH, SH-50-EtOH, DS-Abs-EtOH, HN-Abs-EtOH, and JM-Abs-EtOH, respectively.

3.2.3. *FRAP*

FRAP results are shown in Figure 3. The ferric reducing ability of raw rice extracts was significantly higher than their modified rice ($p < 0.05$). Among the raw rice extracts, significant differences were also observed among varieties of rice. DS-50-EtOH showed the highest EC with values of 4.26 ± 0.26 mM/mg followed by SH-Abs-EtOH, HN-50-EtOH, JM-Abs-EtOH, SH-50-EtOH, DS-Abs-EtOH, HN-Abs-EtOH, and JM-50-EtOH, respectively.

3.3. *Anti-inflammatory activity of rice samples*

All rice extracts and the modified rice at the concentration used did not have a significant cytotoxic effect towards macrophages as determined using MTT assay. Pro-inflammatory cytokines IL-6 and TNF-α were detected using commercial ELISA. The results of anti-inflammatory activity were shown in Figure 4. All pro-inflammatory cytokines exhibited significantly higher production in the positive control than the negative control. From the results, the anti-inflammatory activity of the raw rice extracts was significantly higher than their modified forms. Moreover, the activity of the rice extracts obtained from Abs-EtOH was significantly higher than those obtained from 50-EtOH. Furthermore, the pigmented rice extracts showed higher activity than the white rice extracts. The secretion of IL-6 was significantly reduced by 33.24 ± 2.75% after adding DS-Abs-EtOH and by 60.96 ± 7.27% after adding HN-Abs-EtOH. The secretion of TNF-α was also significantly reduced by 56.48 ± 0.10% after adding DS-Abs-EtOH and by 82.80 ± 0.97% after adding HN-Abs-EtOH. From these results, it can be concluded that DS-Abs-EtOH has

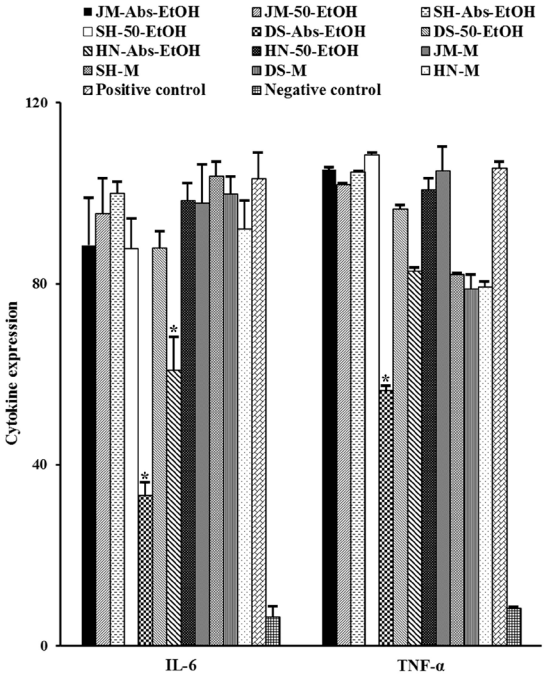

Figure 4. Effects of rice samples on IL-6 and TNF-α expression in LPS-stimulated macrophages (*the inhibitory activity of rice sample on cytokine secretion was significantly reduced at least 25% from a positive control).

the highest anti-inflammatory activity.

4. Discussion

The amount and type of compounds existing in each rice variety is different (*25*). The bioactivities of rice grains are hypothesized that it is according to the constituents existing in them. In this study, four different rice varieties including white rice, JM and SH, as well as pigmented rice, DS and HN, were investigated for their antioxidant and anti-inflammatory activities in comparison with the respective rice after subjecting to the chemical modification. The modified rice used

in the present study was obtained from etherification. Under this chemical reaction, raw rice starch can be modified into carboxymethyl starch (*15*) which can easily dissolve in water. Raw rice powder was found to be water insoluble, therefore extraction with organic solvent was used to collect essential compounds from raw rice samples. Abs-EtOH, containing 99.9% ethanol, was select as a suitable solvent because of its less polarity than water and less toxicity than other organic solvents (*26*). Water-cosolvent systems have been used for extraction of various plant compounds as an alternative to the extraction processes (*27*). Therefore 50-EtOH, containing 50% ethanol, was also selected to use in this study. The obtained rice extracts possessed different color due to rice variety and type of extracting solvent system. Different yield of extracts was found even from the same rice variety, depending on the solubility in the used extracting solvents and amount of the beneficial compounds in each rice grain variety. Extraction of the modified rice using the same manner and same extracting solvent showed that no any extract could be obtained. This might be due to the loss of certain ingredients at the final step of rice modification that the residual reagents and the unwanted products were completely washed out using ethanol. All solutes in the modified rice samples that could be dissolved in ethanol were removed in this step. Therefore, we decided to use the whole part of modified rice in the activity comparison test. To be comparable, the amount of the modified rice sample used was the same as that of the raw rice that gave the extract concentration used in the test.

Many reactions and mechanisms are involved in antioxidant processes. The reactions of antioxidant to decrease free radical are direct and indirect processes. The direct processes are *via* free radical scavenging and metal ion chelating activities. The indirect processes are *via* inhibiting the activity of free radical generating enzymes and enhancing the activity of intracellular antioxidant enzymes (*28*). To demonstrate antioxidant activity by a free radical scavenging mechanism, ABTS and DPPH assay were applied. The principle of ABTS assay is to monitor the decay of the radical-cation of ABTS resulting from the oxidation of ABTS. Radical-cation of ABTS is soluble in both aqueous and organic solvents. This assay is not affected by ionic strength and can be done at different pH levels, therefore it was commonly used (*29*). Another method for detection of antioxidant activity based on free radical scavenging mechanism is DPPH assay. This assay is according to the color changes of the DPPH free radicals. The purple radical DPPH solution was converted to the yellow non-radical DPPH by the antioxidant having electron donating activity. These two methods can determine the free radical scavenging activity of the test samples directly to indicate the antioxidant activity of the samples. These ABTS and DPPH assays were selected to use for antioxidant activity testing in the present study. Moreover, due to the different mechanism of antioxidant action, FRAP assay was used for determination of reducing activity. This method was applied to measure antioxidant activity by determination of the total reducing capacity of the compound based on the ability to reduce Fe^{3+} into Fe^{2+}. Two mechanisms complement one another and give useful information of antioxidant activity (*30*).

From the results of ABTS assay, the highest potential of ABTS free radical scavenging was DS-50-EtOH and HN-50-OH. This result is confirmed by the result from DPPH assay that DS-50-EtOH and HN-50-EtOH also showed the highest potential of DPPH free radical scavenging. The result from FRAP assay reveals that the highest reducing power was obtained from DS-50-EtOH. These results demonstrated that 50-EtOH is the better extracting solvent than Abs-EtOH. This was due to the ability of solvent to dissolve antioxidant compounds in rice grains. Moreover, the results indicated that the pigmented rice grains possess antioxidant activity with respect to the mechanisms of free radical scavenging and reducing activity. Previous studies demonstrated that pigmented rice exhibited higher antioxidant activity than white rice (*31*). This is according to the existing anthocyanins and proanthocyanins or condensed tannins which are the most prevalent phenolic compounds found in the pigmented rice (*19*). Those compounds have ability to donate hydrogen and act as reducing agents (*32*). Rice bioactive compounds such as anthocyanin are water soluble and the existing phenolic compounds are in the soluble and insoluble forms (*30,33*) Therefore, extracting solvent systems also play an important role on the antioxidant activity of rice due to the amount and type of the compounds that can be extracted.

Besides the results of bioactive analysis and antioxidant activity, the *in vitro* anti-inflammatory effect of rice was evaluated with macrophages cells. The effects of white rice and pigmented rice on IL-6 and TNF-α expression were examined for their potential anti-inflammatory activities. From our results, the pigmented rice has a potent anti-inflammatory action. The extracts of DS and HN obtained from Abs-EtOH achieved the inhibition against IL-6. The anti-inflammatory activity of the test samples is accounted when the pro-inflammatory cytokines, such as IL-6 and TNF-α were significantly reduced by at least 25%. In the present study, DS-Abs-EtOH significantly suppressed IL-6 and TNF-α secretion, indicating that DS-Abs-EtOH extract has high potential as an anti-inflammatory agent. To the best of our knowledge, this is the first study which reports the significantly high anti-inflammatory effects of DS, an important pigmented rice variety of Thailand. In previous report from other groups, the Abs-EtOH extracts of two varieties of HN grown in Phayao province showed low anti-inflammatory activity against IL-6, TNF-α, and

nuclear factor-kappa B. Those results contrast to our results that HN-Abs-EtOH in the present study exhibits high inhibition against IL-6. This difference might be due to the variation of different cultivation area that the yield of active compounds obtained is different amount (*13*).

Different polarity of extracting solvents can affect the bioactive composition of the obtained extracts. Previous report from other groups shows that the water extracts of *Chaenomeles sinensis* have higher antioxidant activity whereas the ethanolic extracts have higher anti-inflammatory activity (*34*). Another report demonstrates that the polar fractions of *Suaeda asparagoides* have higher antioxidant activity than the non-polar fractions whereas the non-polar fractions have higher anti-inflammatory activity than the polar fractions (*35*). In the present study, the higher polar rice extracts from 50-EtOH showed higher antioxidant activity than the lower polar extracts from Abs-EtOH, while Abs-EtOH extracts showed higher anti-inflammatory activity than 50-EtOH extracts. These results are in line with the previous reports and that was according to the different bioactive compounds in the extracts.

The present results from antioxidant and anti-inflammatory studies clearly indicate that modification of rice structure has an effect to reduce antioxidant and anti-inflammatory property of rice. It is hypothesized that some bioactive compounds were degraded during the chemical modification process. In our process of rice modification, rice powder was treated with acid, base, and high temperatures. Previous studies reported that phenolic compounds and anthocyanins are labile to heat treatment and unstable under the alkaline pH. These factors induced the degradation and resulting in color change of bioactive compounds (*34*). Moreover, as mentioned above, the moodified rice was washed several times with ethanol at the final step of rice modification. This step also caused the loss of many active ingredients that could be dissolved in the ethanol. This was confirmed by visual observation, that the color of the modified rice was different from the raw rice indicating that some active compounds were lost. In addition, high viscosity of the systems containing modified rice might retard the reaction of the test. According to these factors, DS-M and HN-M showed less activities of antioxidant and anti-inflammatory than their respective raw rice. Our results confirm that after rice modification, the antioxidant and anti-inflammatory activities can be obtained from only pigmented rice variety. From the results of MTT assay, cytotoxicity to the macrophage cells was not found in all rice samples indicating the safety of the samples.

In conclusion, difference in rice varieties leads to the difference antioxidant and anti-inflammatory potential. Chemical modification of rice causes significant reduction on the antioxidant and anti-inflammatory activity of rice. The pigmented rice possesses better antioxidant and anti-inflammatory activities than the white rice. In addition, the results suggest that DS is a promising source of antioxidant and anti-inflammatory compounds.

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