ANTIOXIDANT CAPACITIES, PHENOLIC, ANTHOCYANIN AND PROANTHOCYANIDIN CONTENTS OF PIGMENTED RICE EXTRACTS OBTAINED BY MICROWAVE-ASSISTED METHOD

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Abstract

Pigmented rice has high potential antioxidant activities because it contains high amount of bioactive compounds. The phenolic, anthocyanins and proanthocyanidin from Homnin black rice (HN), and Munpu red rice (MP) were extracted by using microwave-assisted method (MAE) at the power levels of 180, 360, 540, 720, and 900 W with acidified ethanol at ambient temperature for 30 min. Moreover, their antioxidant activities were determined using DPPH radical scavenging activity (DPPH) and ferric reducing power (FRAP) assay. The results indicated that HN extracted with the lowest microwave power level (180 W) showed the highest phenolic (0.89 mg GAE.mL^{-1}), anthocyanins (0.19 mg cyanidin-3-glucoside.mL^{-1}), proanthocyanidins (0.11 mg EPC.mL^{-1}), DPPH radical scavenging (61.29%), and FRAP activity (0.28 mg AAE.mL^{-1}), while MP extracted with the highest microwave power level (900 W) established highest phenolic (1.85 mg GAE.mL^{-1}), anthocyanins (0.03 mg C3G.mL^{-1}), proanthocyanidins (0.33 mg EPC.mL^{-1}), DPPH radical scavenging (56.90%) and FRAP capacity (0.97 mg AAE.mL^{-1}). It appeared that the higher yield of active compounds from MP can be extracted by using higher microwave power level, while extractionofthatfrom HN requires lower microwave power level.

Keywords: Antioxidation activity, extraction, Homnin rice, Munpu rice

Introduction

Pigmented rice have become increasingly interested in antioxidants, mainly due to they was a good source of bioactive compounds such as γ-oryzanol, α-tocopherols, phenolic compounds. The phenolic compounds in pigmented rice has been reported to contain anthocyanins which cyanidin-3-glucoside was a major in black rice (Osawa, 1999), proanthocyanidins which catechin was a major in red rice (Nawa and Ohtani, 1992)

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and other phenolics (Yawadio *et al*., 2007). Antioxidant activities of the color pigment in aleurone layer of rice have been demonstrated by Hu *et al*. (2003); Ichikawa *et al*. (2001); Oki *et al*. (2002). In Thailand, several pigmented rice varieties have been cultivated and consumed, especially black and red rice. Homnin black rice (HN) and Munpu red rice (MP) were well known as commercial pigmented rice cultivarand possess many active compounds with antioxidant activities. Therefore, effective extraction of these active phenolic compounds from pigmented rice can be utilized for developing functional foods, cosmetics and in nutraceutical and pharmaceutical and health products.

Microwave is an electromagnetic radiation with a frequency form 0.3 to 300 GHz and is alternatively extraction method which is inexpensive, simple, shorter extraction time, and higher extraction yield and selectivity by affecting the polar molecules in extraction solvent and increase the internal pressure of the solid material (Orsat and Raghavan, 2005; Alupului *et al*., 2012). Hence, almost extraction using microwave were focus on phenolic compounds from plants such as curcumin, longan, and rosmarry which increased extraction yield of total phenolics with significant reduction in extraction time and solvent consumption (Kaufmann and Christen, 2002; Mandal *et al*., 2008; Pan *et al*., 2008; Proestos and Komaitis, 2008). However, extraction of the active compounds from HN and MP rice by using of MAE have not been reported previously. Thus, the aim of this study was to determine the effect of the power levels of MAE to extraction efficacy of active compounds from HN and MP rice by determining of the content of phenolics, anthocyanins and proanthocyanidins. Moreover, their antioxidant activities were also establishedusing DPPH radical scavenging activity (DPPH) and ferric reducing power (FRAP) assays.

Materials and Methods

Plant Materials and Chemicals

Two Thai rice varieties were involved in this study. Homnin black rice was cultivated in Chiang Mai which was sold under the Tai Tai organic brand. Man pu red rice was cultivated in Prachinburi whichwas sold under the Nature zone brand. Hydrochloric acid (HCl) was purchased from J.T Baker. Ethanol, sulfuric acid, and trichloro acetic acid were purchased from Merck. 1,1-dipheny l-2-picrylhydrazyl (DPPH), cyanidin-3-glucoside, epicatechin, folin-ciocalteu reagent, gallic acid, sodium acetate, sodium carbonate, and vanillin were purchased from Sigma. Ferric chloride, and potassium ferricyanide was purchased from Fisher scientific.

Microwave-Assisted Extraction (MAE)

The MAE of rice samples were extracted with of 1% HCl in ethanol at ratio 1:2 under microwave oven (Severin, Germany) at various powers (180, 360, 540, 720, and 900 W) for 30 min. The mixtures were filtered through Whatman filter paper No.1 and then stored at 4^{o}C until analysis.

Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) was analyzed using the Folin-Ciocalteu assay (Gajula *et al*., 2009). Briefly, the 20 μL of extracts were mixed with 20 μL of Folin-ciocalteu reagent, 125 μL of 7% Na_2CO_3 and 50 μL of DI water. The mixture was incubated at room temperature for 90 min and then measured the absorbance at 750 nm using microplate reader (Biochrom, USA). The TPC were expressed as mg gallic acid equivalents (GAE) per mL extract.

Determination of Total Anthocyanins Content (TAC)

The total anthocyanins content (TAC) was determined by using the pH-differential method described (AOAC Official, 2005). The extracts (20 μL) were mixed with 180 μL of the pH 1.0 and 4.5 buffers and measured absorbance at 520 and 700 nm by microplate reader (Biochrom, USA). The TAC were expressed as cyanidin-3-glucoside equivalents (mg C3G.mL^{-1} extract) by calculated using Equation 1:

$$Total\ anthocyanins\ (mg/L) = A \times MW \times DF \times 1000 / (\varepsilon \times 1) \quad (1)$$

where A is $(A_{520} - A_{700})_{pH=1.0} - (A_{520} - A7_{00})_{pH=4.5}$, MW is molecular weight, DF is dilution factor, 1 is path length in cm, 1000 is conversion from g to mg and ε is molar extinction coefficient for C3G (26900 $L.mol^{-1}.cm$).

Determination of Total Proanthocyanidin Content (TPAC)

The total proanthocyanidin content (TPAC) was quantified using the Vanillin assay (Nakamura *et al*., 2003). Briefly, the 20 µL of extracts were added with 100 µL of 1% vanillin in sulfuric acid. The mixture was incubated at room temperature for 15 min and determined the absorbance at 500 nm by using microplate reader (Biochrom, USA). The results were expressed as epicatechin equivalent (mg $EPE.mL^{-1}$ extract).

DPPH Radical Scavenging Activity Assay (DPPH)

The scavenging activity of 1, 1-diphenyl -2-picrylhydrazyl (DPPH) free radicals was determined according to Rangkadilok *et al*. (2005). The 5 µL of extract was added with 195 µL of 0.1 M DPPH solution. The mixture was stand at room temperature for 30 min and measured absorbance at 515 nm using microplate reader (Biochrom, USA). The DPPH scavenging activity was calculated following Equation 2:

$$DPPH\ scavenging\ activity\ (\%) = [(A_{control}-A_{sample})/A_{control}] \times 100 \quad (2)$$

Ferric Reducing Power Assay (FRAP)

The ferric reducing power (FRAP) was determined according to the ferric reducing power method (Takashi and Toshihiro, 2009). The extracts (25 µL) were added with 50 µL of 1% $K_3Fe(CN)_6$ and stand at room temperature for 60 min. Then, the mixture was added 25 µL of 10% trichloro acetic acid and 75 µL of DI water. The absorbance was measured at 700 nm using microplate reader (Biochrom, USA) as absorbance 1 (A1). Then, the mixture was added 25 µL of 0.1% $FeCl_3$ and the absorbance was measured at 700 nm again as absorbance 2 (A2). The optical density (OD) of sample was expressed as ascorbic acids equivalent (mg $AAE.mL^{-1}$ extract) and calculated follows as the Equation 3:

$$OD = (A2-A1)_{sample} - (A2-A1)control \quad (3)$$

Statistical Analysis

The obtained data were statistically analyzed using the SPSS program version 11.5 for window (SPSS Inc, Chicago, IL, USA) and the differences were considered significant when $p < 0.05$.

Results and Discussion

HN and MP rice were long grain rice with different color in aleurone layer, HN appeared to beblack whereas MP represented to be darkred. In preliminary screening of method, the samples were extracted by three extraction techniques, shaking, sonicate, and MAE (540 W) and measured for their total phenolic containg (TPC). MAE showed the highest extraction efficacy of TPC from both HN and MP rice followed by shaking and sonication methods. Amongst HN extract, micro wave produced 4 and 2 times higher TPC than shaking and sonication, respectively. For MP extract, microwave also produced 1.5 folds higher TPC than shaking and sonication extraction. Therefore, MAE was afterward investigated the optimal microwave power level (180-900 W) to extract active compounds from HN and MP rice.

The HN and MP extracts seemed to be red-brown color. MP extract appeared to be brighter yellow shade than that of HN extracts. The TPC, TAC, TPAC and antioxidant activities of HN and MP extracts were reported in Table 1. MP showed significant higher TPC (with a mean 1.75 mg $GAE.mL^{-1}$) than that of HN (with a mean 0.79 mg $GAE.mL^{-1}$). There was significantly different from the TAC and TPAC of both pigmented rice. HN (with a mean 0.12 mg $C3G.mL^{-1}$) was higher TAC than MP (with a mean 0.02 mg $C3G.mL^{-1}$)

where as, MP (with a mean 0.31 mg EPE.mL^{-1}) showed higher TPAC than HN (with a mean 0.09 mg EPE.mL^{-1}). These results was similar to the previous studies showing that black rice found higher anthocyanin content than that of red rice, whereas the red rice had higher proanthocyanidin content than that of black rice (Min *et al.,* 2012).

The antioxidant activities of microwave-assisted HN and MP extract were determined by DPPH radical scavenging activity (DPPH) and ferric reducing power (FRAP) assays. The DPPH inhibitions of HN and MP were not significant different with range from 51.47 to 61.29%. The highest activity was found in HN extract at 180 W. The FRAP of MP was significantly higher FRAP than HN which was in the range of 0.25–0.28 mg AAE.mL^{-1} for HN and 0.91–0.97 mg AAE.mL^{-1} for MP.

The power level of microwave affected the TPC, TAC, TPAC, and antioxidant activities of both rice in different tendency. The TPC, TAC, TPAC, and antioxidant activities of MP were increased with the increase of microwave power, while that of HN were decreased with the increase of microwave power. The different effect of microwave power level may due to different active compounds in HN and MP and their thermal degradation resistance. These results may be due to during extraction of substance from plants by microwave, the energy rapidly delivers to plant matrix and is efficiently absorbed by some substances inside plant materials, especially the polar molecules and converted into thermal energy. Consequently, the internal temperature of the plant cells increases drastically (Rostagno *et al.*, 2009; Zhang *et al.*, 2011). Although temperature is a significant factor influencing the rate of extraction and extraction yield, the overheating can degrade thermal sensitivity substance. Moreover, microwave irradiation may accelerate the chemical structure or changes of some compounds (Zhao *et al.*, 2006; Ghani *et al.*,

Table 1. The total phenolic, anthocyanin, proanthocyanidin contents and antioxidant activities of Homnin and Munpu rice extracts

Samples	Micro wave power (W)	TPC (mg GAE.mL^{-1})	TAC (mg C3G.mL^{-1})	TPAC (mg EPE.mL^{-1})	DPPH (% inhibition)	FRAP (mg AAE.mL^{-1})
Homnin black rice (HN)	180	0.89 ± 0.00^{c}	0.19 ± 0.01^{a}	0.11 ± 0.02^{c}	61.29 ± 0.78^{a}	0.28 ± 0.02^{c}
	360	0.86 ± 0.07^{c}	0.16 ± 0.02^{a,b}	0.09 ± 0.03^{c,d}	59.01 ± 0.29^{a,b}	0.25 ± 0.01^{c}
	540	0.81 ± 0.06^{c}	0.12 ± 0.01^{b}	0.08 ± 0.01^{c,d}	54.79 ± 0.25^{c,d}	0.24 ± 0.02^{c}
	720	0.76 ± 0.03^{c}	0.08 ± 0.04^{c}	0.08 ± 0.01^{c,d}	54.45 ± 0.61^{c,d}	0.24 ± 0.01^{c}
	900	0.63 ± 0.12^{d}	0.07 ± 0.04^{c}	0.07 ± 0.01^{d}	51.47 ± 3.84^{d}	0.23 ± 0.02^{c}
	Average	0.79 ± 0.11	0.12 ± 0.05	0.09 ± 0.02	56.20 ± 3.92	0.25 ± 0.02
Mun pu red rice (MP)	180	1.61 ± 0.01^{b}	0.01 ± 0.01^{d}	0.30 ± 0.01^{b}	54.48 ± 0.16^{c,d}	0.89 ± 0.04^{b}
	360	1.66 ± 0.00^{b}	0.01 ± 0.02^{d}	0.30 ± 0.00^{b}	55.69 ± 0.82^{b,c}	0.89 ± 0.02^{b}
	540	1.79 ± 0.03^{a}	0.02 ± 0.04^{d}	0.31 ± 0.00^{a,b}	56.60 ± 2.29^{b,c}	0.90 ± 0.10^{a,b}
	720	1.81 ± 0.05^{a}	0.03 ± 0.00^{d}	0.32 ± 0.01^{a,b}	56.90 ± 0.04^{b,c}	0.91 ± 0.03^{a,b}
	900	1.85 ± 0.01^{a}	0.03 ± 0.02^{d}	0.33 ± 0.01^{a}	56.90 ± 0.04^{b,c}	0.97 ± 0.01^{a}
	Average	1.74 ± 0.10	0.02 ± 0.01	0.31 ± 0.02	56.12 ± 1.28	0.91 ± 0.05

[*]*(Mean ± S.D., n=3); Different letters in the same column indicate significantly different at $p \leq 0.05$.*

2008; Wang *et al*., 2008) which then result in the reduction of extraction yield.

The anthocyanins, malvidin, pelargonidin-3, 5-diglucoside, cyanidin-3-glucoside and cyanidin-3,5-diglucoside, was found to be major substances in black rice were (Zhang *et al*., 2006). These compounds degrade at high temperature and lead to brown products, especially in the presence of oxygen (Markakis, 1982; Lin and Chou, 2009; Rodrigues *et al*., 2009; Sadilova *et al*., 2009; Jiménez *et al*., 2010). Thus the application of high microwave power level generated heat that may cause the decomposition of active substance and antioxidant activities for HN. On the other hand, themajor constituents in red rice was proanthocyanidins such as catechins and epicatechins (Nawa and Ohtani, 1992; Oki *et al*., 2002) which higher thermal stability than anthocyanins (Goto-Yamamoto *et al*., 2010). This results suggested that the best extraction condition for active compounds from MP required the high microwave power level (900 W), while extractionofthatfrom HN required low microwave power level (180 W).

Conclusions

Microwave-assisted extract was currently regarded as a robust alternative to traditional extraction techniques. The optimal power level for extraction of pigmented rice depended on their major substances of rice varieties. The active compounds of MP were high heat resistance and can be more efficiently extracted when using high microwave power level. In contrast,the active compound from HN rice was thermolabile and degraded by high microwave power level.

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References

Aluplui, A., Călinescu, I., and Lavric V. (2012). Microwave extraction of active principles from medicinal plants. U.P.B. Sci. Bull. Series. B., 74:129-142.

AOAC Official. (2005). Total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines. J. AOAC Int, 88:1269.

Gajula, D., Verghese, M., BoatengJ., and Walker, L.T. (2009). Determination of total phenolics, flavonoids and antioxidant and chemopreventive potential of basil (*Ocimum basilicum* L. and *Ocimum tenuiflorum L.*) Int. J. Cancer Res., 5(4):130-143.

Ghani, S.B.A., Weaver, L., Zidan, Z.H., Ali, H.M., Keevil, C.W., and Brown, R.C.D. (2008). Microwave-assisted synthesis and antimicrobial activities of flavonoid derivatives. Bioorg. Med. Chem. Lett., 18:518-522.

Goto-Yamamoto, N., Mori, K., Numata, M., Koyama, K., and Kitayama, M. (2010). Effects of temperature and water regimes on flavonoid contents and composition in the skin of red-wine grapes. J. Int. Sci. VigneVin. special issue Macrowine. p. 75-80.

Hu, C., Zawistowski, J., Ling W., and Kitts, D.D. (2003). Black rice (Oryza sativa L. indica) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. J Agr. Food, Chem., 51:5271-5277.

Ichikawa, H., Ichiyanagi, T. Xu, B., Yoshii, Y., Nakajima, M., and Konnichi, T. (2001). Antioxidant activity of anthocyanins extract from purple black rice. J. Med. Food 4(4):211-218.

Jiménez, N., Bohuon, P., Lima, J., Dornier, M., Vaillant, F., and Pérez, A. M. (2010). Kinetics of anthocyanin degradation and browning in reconstituted blackberry juice treated at high temperatures (100-180°C). J. Agr. Food, Chem., 58:2314-2322.

Kaufmann, B. and Christen, P. (2002). Recent extraction techniques for natural products: microwave-assisted extraction and pressurised solvent extraction, Phytochem. Analysis, 13(2): 105-113.

Mandal, V., Mohan, Y., and Hemalatha, S. (2008). Microwave assisted extraction of curcumin by sample-solvent dual heating mechanism using Taguchi L9 orthogonal design. J. Pharm. Biomed. Anal. 46: 322-327.

Markakis, P. (1982). Stability of anthocyanins in foods. In P. Markakis (Ed.), Anthocyanins as food colors Academic Press Inc. NY, p. 163-178.

Min, B., Gu, L., McClung, A.M., Bergman, C.J., and Chen, M.H. (2012). Free and bound total phenolic concentrations, antioxidant capacities, and profiles of proanthocyanidins and anthocyanins in whole grain rice (*Oryza sativa* L.) of different bran colours. Food Chem, 133:715-722.

Nakamura, Y., Tsuji, S. and Tonogal, Y. (2003). Analysis of proanthocyanidins in grape seed extracts, health foods and grape seed oils. J. Health Sci., 49(1):45-54.

Nawa, Y. and Ohtani, T. (1992). Property of pigments in rice hulls of various colors. Food Industry, Tokyo, 11:28-33.

Oki, T., Masuda, M., Kobayashi, M., Nishiba, Y., Furuta, S., Suda, I., and Sato, T. (2002). Polymeric procyanidins as radical-scavenging components in red-hulled rice. J. Agr. Food Chem., 50:7524-7529.

Orsat, V. and Raghavan, V.(2005). Microwave technology for food processing: an overview. In: The Microwave Processing of Foods. Schuber,H. and Regier, M., (eds.). Woodhead Publishing Cambridge, England, p. 105-115.

Osawa, T. 1999. Protective role of rice polyphenols in oxidative stress. Anticancer. Res. 19: 3645-3650.

Pan, Y., Wang, K., Huang, S.,Wang, H., Mu, X., He, C., Ji, X., Zhang, J., and Huang, F. (2008). Antioxidant activity of microwave-assisted extract of longan (*Dimocarpus Longan* Lour.) peel. Food Chem., 106:1264-1270.

Proestos, C., & Komaitis, M. (2008). Application of microwave-assisted extraction to the fast extraction of plant phenolic compounds. LWT-Food Sci. Tech., 41:652-659.

Rangkadilok, N., Sitthimonchai, S., Worasuttayangkurn, L., Mahidol, C., Ruchirawut,M., and Satayavivad, J. (2007). Evaluation of free radical scavenging and antityrosinase activities of standardized longan fruit extract. Food Chem. Toxicol., 45:328-336.

Rodrigues, A.S., Pérez-Gregorio, M.R., García-Falcó n, M.S., and Simal-Gíndara, J. (2009). Effect of curing and cooking on flavonols and anthocyanins in traditional varieties of onion bulbs. Food Res. Inter., 42:1331-1336.

Rostagno, M.A., Villares, A., Guillamón, E., García-Lafuente, A. and Martínez, J.A. (2009). Sample preparation for the analysis of isoflavones from soybeans and soy foods. J. Chromatogr. A., 1216:2-29.

Sadilova, E., Stintzing, F.C., Kammerer, D.R., and Carle, R. (2009). Matrix dependent impact of sugar and ascorbic acid addition on color and anthocyanin stability of black carrot, elderberry and strawberry single strength and from concentrate juices upon thermal treatment. Food Res. Inter., 42:1023-1033.

Takashi, K., and Toshihiro, Y. (2009). Changes of radical-scavenging capacity and ferrous reducing power in chub mackerel Scomber japonices and Pacific saury Cololabis saira during 4°C storage. LWT Food Sci. Tech., 42(6):1070-1075.

Wang, L. and Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. Trends Food Sci. Technol. 17:300-312.

Wang, Y., You, J., Yu, Y., Qu, C., Zhang, H., Ding, L., Zhang, H., and Li, X. (2008). Analysis of ginsenosides in Panax ginseng in high pressure microwave-assisted extraction. Food Chem., 110:161-167 .

Yawadio, R., Tanimori, S., and Morita, N. (2007). Identification of phenolic compounds isolated from pigmented rices and their aldose reductase inhibitory activities. Food Chem., 101:1616-1625.

Zhang, M.W., Guo, B.J., Zhang, R.F., Chi, J.W., Wei, Z.C., Xu, Z.H., and Tang, X.J. (2006). Separation, purification and identification of antioxidant compositions in black rice. Agric. Sci. China., 5:431-440.

Zhang, H. F., Yang, X.H. and Wang, Y. (2011). Microwave assisted extraction of secondary metabolites from plants: Current status and future directions. Trends Food Sci. Technol., 22:672-688.

Zhao, L., Zhao, G., Chen, F., Wang, Z., Wu, J., and Hu, X. (2006). Different effects of microwave and ultrasound on the stability of (all-E)-astaxanthin. J. Agr. Food Chem., 54:8346-8351.