Journal of Food Science and Agricultural Technology

International peer-reviewed scientific online journal

Published online: http://jfat.mfu.ac.th

Original Research Article

Effect of dry heating temperature and time on radical scavenging activities and bioactive compounds in black rice varieties (*Oryza sativa* L. indica)

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ARTICLE INFO

Article history:

Received 30 September 2014

Received in revised form 19 December 2014

Accepted 28 January 2015

Keywords:

Bioactive compound

Black rice varieties

Dry heating temperature

Radical scavenging activities

ABSTRACT

The objective of this research was to investigate the effect of dry heating temperature and time on radical scavenging activities and bioactive compounds in black rice (*Oryza sativa* L. (indica)) kernels. The experimental design as response surface methodology (RSM) was used to determine the optimum temperature and time. Black rice kernels 25 samples were dry heated at different temperatures (80, 100, 120, 140 and 160°C) and different time (10, 15, 20, 25 and 30 min) and subsequently extracted with 95% ethanol respectively. The antioxidant activity values EC50 determined by 2-diphenyl-1-picrylhydrazyl (DPPH) assays at low temperature were 165.89 g flour/g DPPH and EC50 decreased when increasing temperature. Also the total monomeric anthocyanins content from 65.65 mg/L was decreased to 12.58 mg/L, but high total phenolics contents increased when increasing temperature from 72.0941 to 87.9941 GAE mg/g crude extract. Free sugar content was analysed by HPLC and decreased from 1746.91 to 66.43g/100 mL when increasing temperature. The results showed overall values of the black rice varieties were significantly affected by the dry heating temperature and that was important factor affected to the quality of the black rice varieties. The contour plots indicated that the optimum dry heating temperature and time were 120 °C and 25 min, respectively.

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Published by School of Agro-Industry, Mae Fah Luang University

ISSN: 2408-1736

INTRODUCTION

Black Rice Varieties (*Oryza sativa* L. indica) has been comsumed for a long time in Asia, especially Korea, Japan, China and many countries in Southeast Asia (Kanitha Tananuwong and Wanida Tewaruth, 2010). Several varieties of rice contained pigment particularly in brown, red and black rice, have been widely cultivated in Thailand and generally used as an ingredient in snack, dessert, wine, food supplement, phamaceutical and cosmetic (Kanitha Tananuwong and Wanida Tewaruth, 2010; Jaranjit Phengrat and Suwat Jearakongman, 2010). It has been reported that pigment in rice kernel is source of bioactive compound such as anthocyanins and phenolic compound which have benefit for health due to their antioxidant activities and radical scavenging activities (R. Sompong *et al*., 2010).

In the studies of bioactive compound in black rice, it was found that phenolic compounds in plants exist either in the free form or the bound form. Generally phenolic compounds present in rice grains as phenolic acid and flavonoids found in cereals, fruits and vegetables (Jitlada Vichapong *et al*., 2010). Anthocyanin is found as secondary metabolite in black rice, especially as cyanidin-3-O-glucoside and peonidin-3-O-glucoside (Gema Pereira-Caro *et al*., 2013; Sutharut, J and Sudarat, J., 2012). Anthocyanin is unstable and can be destroyed by factors such as pH, light, thermal treatment, enzymes, ascorbic acid and sulfur dioxide. Preventing of anthocyanin degradation is an important aspect benefit to consumers; therefore, thermal degradation of anthocyanin is a major problem in food industry. (Zhao Hou *et al*., 2013).

In food industry, heating is used to improve and alter food quality, extend the shelf-life and improve the processing efficiency of subsequent treatment such as dry heating, hydrothermal treatment, microwave heating, extrusion and γ-irradiation. Effects on the product are decisively caused by the heating conditions. The most important conditions of the heating process are the temperature and time. (Kwang-Sup Youn and Hun-Sik-Chung, 2012; Sung-Min Kim *et al*., 2014). Amonrat Thanonkaew *et al*. (2012) reported that extrusion (dry heat) has been ideal for stabilization because this process prevents additional moisture and it also helps eliminate excess moisture. Temperatures used for the stabilization varied from 100 to 140 °C. Therefore, stabilization of rice bran depends on temperature, duration of heat treatment, moisture content, pH, and other parameters.

From the studies using black rice as raw material, it has been focused on temperature and heating which may cause changes in Maillard reaction. Therefore, the objective of this research was to investigate the effect of dry heating temperature and time on radical scavenging activities and bioactive compounds in black rice (*Oryza sativa* L. (indica)) kernels to find the optimum dry heating temperature and time.

MATERIALS AND METHODS

Materials

Selected black rice (*Oryza sativa* L. (indica)) kernels cv. Kum Doi Muser which The cultivation in the north of Thailand, because of the high nutritional value, widely consumed, where to buy it and affordable. The bought at Tesco Lotus department store prices 148 bath which used in this study. Then, the black rice kernels were tray dried at temperature 60 °C until the moisture is constant (about 8%) and subsequently packed in polyethylene vacuum bag size was 7×11 inch of 25 samples.

Experimental design and statistical analysis

The dry heating temperatures and times were selected according to a central composite design (CCD). The independent variables, temperature (X_1) and time (X_2), varied from 80 to 160 °C and from 10 to 30 min respectively, at surrounding conditions of dry heating temperature under open roasting. The ranges of temperature and time are same as commonly used in conventional dry heating. Each independent variable had five levels: -2, -1, 0, +1 and +2. Thirteen combinations were randomly chosen according to a CCD configuration for 2 independent variables (Youn and Chung, 2012). The experimental design of the coded and actual levels of the variables is shown in Table 1.

Table 1 The central composite experimental design employed for the dry heating process of black rice kernels.

Experimental number	Temperture (°C, X_1)		Time (min, X_2)	
	Coded	Actual	Coded	Actual
1	-1	100	0	20
2	1	140	0	20
3	0	120	2	30
4	1	140	2	30
5	0	120	1	25
6	-1	100	2	30
7	0	120	1	25
8	0	120	1	25
9	0	120	1	25
10	1	140	1	25
11	-1	100	1	25
12	0	120	1	25
13	0	120	0	20

Dry heating and extraction process

Black rice kernels (500 g) were dry heated with Coffee Roaster was heat from the coil using electrical system (Bonami Bona Coffee, Thailand) under the conditions selected for each experimental. The kernels were cooled by put into the tray and aerate to cool at temperature 37 °C and samples were ground using mill and sieved to size of 0.84 mm. The mixture of ground kernels 20 g with 200 mL of ethanol 95% were boiled in water bath with shaker at temperature 32 °C for 4 hr. The extract was filtered through Whatman No.4 paper by vacuum pump (R-5-oil recirculating Rotary Vane Vacuum pump) at 3.6-1,920 m^3/cm, 0.1-2.0 millibar and evaporated with rotary vacuum evaporator (BUCHI Rotavapor R-200) at temperature 78 °C for 20 min until the final concentration was obtained at 50 mL volume (Tewaruth, 2007).

Analysis of phenolic compounds

Total phenolic content was determined by the method of Folin-Ciocalteu assay (Tananuwong and Tewaruth, 2010). The 0.1 mL of extract sample was taken to mix with 7.9 mL of distilled water, then added 0.5 mL of Folin- Ciocalteu agent and incubated for 30 seconds at temperature 37°C. Add 1.5 mL of sodium carbonate and incubated for 30 min at room temperature in dark. The absorbance was measured by a spectrophotometer (Spectronic Ò 20 genesys Ô) at 765 nm. The total concentration of phenolic compound was calculated from gallic acid standard curve. The results were expressed as GAE mg/g crude extract.

Determination of total monomeric anthocyanins

Total monomeric anthocyanin content was determined by pH differential method (Tananuwong and Tewaruth, 2010). The absorbance was measured at pH 1.0 and 4.5. The crude extract was diluted with pH 1.0 potassium chloride buffer and pH 4.5 sodium acetate buffer, respectively. The 0.5 mL of crude extract was taken to add 10 mL of buffer and incubated for 15 min. The absorbance was measured by a spectrophotometer (Spectronic Ò 20 genesys Ô) at 510 nm and 700 nm. The total monomeric anthocyanin content of crude extract was calculated in terms of cyaniding-3-glucoside. The concentration of monomeric anthocyanin pigment was calculated by the following equation:

$$\text{Monomeric anthocyanin pigment (mg/l)} = [A_{diff} \times MW \times DF \times 1000] / \varepsilon \quad (1)$$

Where MW represents molecular weight of cyaniding-3-glucoside (449.2), DF is the dilution factor (20), e is molar absorptivity of cyaniding-3-glucoside (26,900 l/mol cm) and A_{diff} was calculated from the following equation:

$$A_{diff} = [(A_{510}\text{-}A_{700})pH1.0] - [(A_{510}\text{-}A_{700})pH4.5] \quad (2)$$

DPPH radical scavenging activity assay

DPPH assay was an adapted from Tananuwong and Tewaruth(2010) with some modifications. The dilution was prepared by using 0.05 mL of the crude extract mixed with 0.95 mL of 3×10^{-5} M DPPH solution and held for 30 min at temperature 37°C. The absorbance was then read at 515 nm. EC50 is the concentration of the antioxidant that caused the decrease of DPPH radicals from the equivalent amount of the sample in DPPH solution (g flour/g DPPH). The radical scavenging activity (%) was calculated from equation:

$$\text{Radical scavenging activity (\%)} = [1 - (A_{sample}/A_{reagent\ blank})] \times 100 \quad (3)$$

Analysis of free sugar content

The content of free sugar was analyzed by HPLC (HPLC pump model 626) adapted from (Youn and Chung, 2012). The substances extracted with ethanol 95% were passed through Sep-Pak C_{18} cartridges (Waters) and 0.45 µm membrane filters and injected into the HPLC using acetonitrile : water (69 : 31, v/v) as the mobile phase at flow rate 4 mL/min with Evaporative light scattering detector. The peaks were quantified by the external standard method. The content of free sugar was defined as the sum of sucrose, glucose and fructose contents.

RESULTS AND DISCUSSION

Statistical analysis

The experimental values of total phenolics, total monomeric anthocyanin, EC50 and total free sugar of dry heated black rice were shown in Table 2. The dry heating temperature and time affected to total phenolics and total monomeric anthocyanin, EC50 and total free sugar significantly ($P < 0.05$). The regression coefficient (R^2) showed more than 0.9 ($P < 0.05$) in every parameter except EC50. Results indicated R^2 for all responses and the significant scores of dry heating temperature and time effected on total phenolics, total monomeric anthocyanin, EC50 and total free sugar of black rice. In Table 3 showed relationship between dry heating condition affected overall values of bioactive compounds, it showed that dry heating temperature and time affected to the contents of total phenolics, total monomeric anthocyanin, EC50 and total free sugar significantly, due to dry heating condition of temperature and time as a result quality changes in black rice. Therefore dry heating temperature and time affected overall values of bioactive compounds in black rice at $P < 0.05$ (Youn and Chung, 2012; Senklang and Anprung, 2010).

Effects of dry heating temperature and time

The response surfaces for total phenolics, total monomeric anthocyanin, EC50 and total free sugar of dry heated black rice were shown in Figure 1.

Phenolic compounds are components commonly found as important elements with biology and functional properties in terms of food quality (Youn and Chung, 2012; Sinha *et al*., 2009). The content of phenolic compounds in black rice increased when increasing dry heating temperature and time (figure 1). The level of phenolic compounds is correlated with dry heating temperature and time significantly ($P < 0.05$). When the levels of dry heating temperature and time were 140 °C and 25 min, the values of total phenolics were 78.6941 GAE mg/g crude extract. The increased production of phenolic compounds during dry heating may be caused by heat which could break the covalent bonds of polymerized polyphenols and subsequently transform high molecular phenolics into low molecular ones. It might also relate to the increased generation of the Maillard reaction during dry heating. This changed the chemical composition by adding of a phenolic molecular into the existing natural structure, leading to formation of the phenolic component in black rice (Wanyo *et al*., 2014).

Total monomeric anthocyanin was pigment in plants as natural color substance that belongs to the flavonoid group called anthocyanins. With positive health effects, the pigment presents in the bran layer of rice (Jiapong and Jiamyangyuen, 2012). The total monomeric anthocyanin contents of black rice decreased when dry heating temperature and time increased (Figure 1). The level of total monomeric anthocyanin is correlated with dry heating temperature and time significantly ($P < 0.05$). When the levels of dry heating temperature and time were 100 °C and 25 min, the values of total monomeric anthocyanin were 28.39 mg/L. The reduced amounts of total monomeric anthocyanin caused by heat that breaking the bonds and forming other compounds, consequently total monomeric anthocyanin content was decreased (Patras *et al*., 2010).

Table 2 Experimental data for response parameters of black rice in relation to dry heating conditions

Experimental number	Total phenolics (GAE mg/g crud extract)	Total monomeric anthocyanin (mg/L)	EC50 (g flour/g DPPH)	Total free sugar (g/100 ml)
1	78.2941	51.10	147.72	1746.91
2	81.9941	29.72	85.49	153.72
3	73.8941	43.20	145.91	467.95
4	77.4941	18.15	98.42	66.43
5	76.7941	44.20	132.01	620.67
6	74.9941	63.65	165.89	1075.15
7	76.7941	44.20	132.01	620.67
8	76.7941	44.20	132.01	620.67
9	76.7941	44.20	132.01	620.67
10	78.6941	12.58	110.24	68.41
11	72.0941	28.39	159.19	1058.61
12	76.7941	44.20	132.01	620.67
13	76.8941	35.73	139.69	832.91

Table 3 Regression coefficient (R^2) the relationship between dry heating conditions and quality changes in black rice

Coefficient	Total phenolics	Total monomeric anthocyanin	EC50	Total free sugar
A	20*	-43.11*	-10.30	-1422.11*
B	6.39	16.57*	-10.32	-272.75
A^2	13.49*	38.62*	-42.67*	758.67*
B^2	2.24	14.96*	-1.09	76.60
AB	15.88*	-24.96*	-0.64	2.151E+006
R^2	0.9012	0.9628	0.8451	0.9063

A = temperature, B = time, * Significant at $P < 0.05$

A2 = temperature2, B2 = time2, AB = coefficient of interaction between temperature and time

DPPH radical scavenging activity was tested by measuring EC50 (Effective Concentration; EC50) of black rice under different dry heating conditions. EC50 values increased during the beginning temperature and time at 80 to 100 °C and 10 to 30 min, after that decreased during the temperature and time at 120 to 160 °C and 10 to 30 min (Figure 1). The level of EC50 is correlated with dry heating temperature and time significantly ($P < 0.05$). When the levels of dry heating temperature and time were 120 °C and 25 min, EC50 values were 132.01 g flour/g DPPH. However, lower EC50 values lead to higher antioxidant efficiency (Tananuwong and Tewaruth, 2010).

Total free sugar content was generally considered as an indirect measuring of the substrate concentration of the non-enzymatic browning reaction or of the nutrients remaining after the browning reaction. Generally, the content of total free sugar decreased with increasing dry heating temperature and time (Figure 1). The level of total free sugar content was correlated with dry heating temperature and time significantly ($P < 0.05$). When the levels of dry heating temperature and time were 120 degree Celsius and 20 min, the total free sugar contents were 823.91 g/100 mL. These results suggested that higher temperature and longer dry heating time will cause many nutrients including carbonyl and amino acid compounds, decomposed in the non-enzymatic browning reactions during the dry heating process (Youn and Chung, 2012).

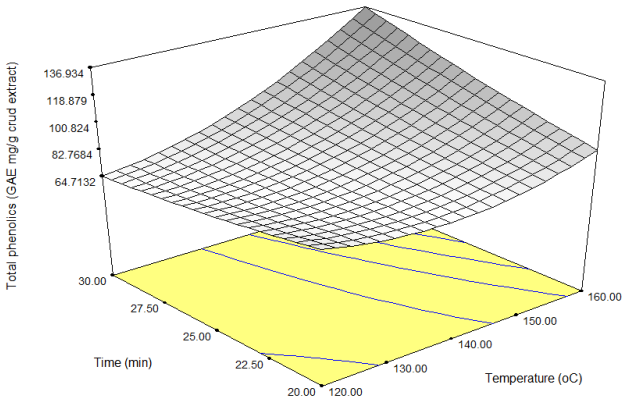
Optimization of dry heating conditions

The optimum dry heating temperature and time were obtained by superimposing the contour plots of the surface responses as shown in Figure 2 for a region of the optimum dry heating conditions of black rice. The optimum area of superimposed contour plots were determined by using the total phenolics, total monomeric anthocyanins, EC50 and total free sugar contents. The results show that the optimum dry heating temperature and time for black rice were 120 °C and 25 min, respectively.

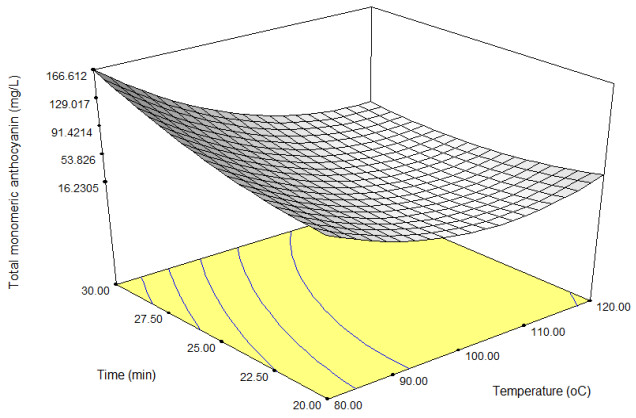
CONCLUSION

The temperature and time for dry heating of black rice extracted with ethanol 95% were studied to investigate the effect on total phenolics, total monomeric anthocyanins, EC50 and total free sugar contents with the experimental design as response surface methodology

(RSM). It was found that contents of total monomeric anthocyanins, EC50 and total free sugar decreased when increasing temperature and time, except content of total phenolics increased when increasing temperature and time. The dry heating temperature and time affected to all values at $P < 0.05$, showing that the overall values can be correlated with dry heating conditions. The optimum dry heating temperature and time obtained graphically using contour plots were 120 °C and 25 min respectively.

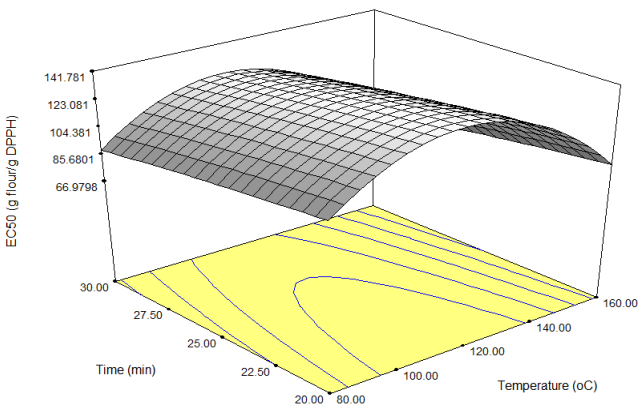


(A)

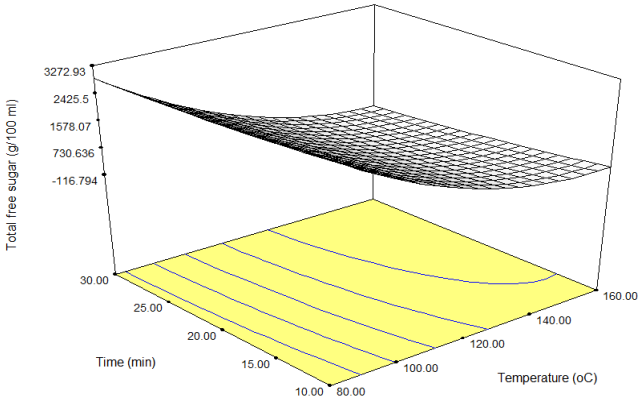


(B)

Figure 1 Response surfaces for total phenolics (A) and total monomeric anthocyanins (B) of black rice as a function of dry heating temperature and time.



(C)



(D)

Figure 2 Response surfaces for EC50 (C) and total free sugar (D) of black rice as a function of dry heating temperature and time

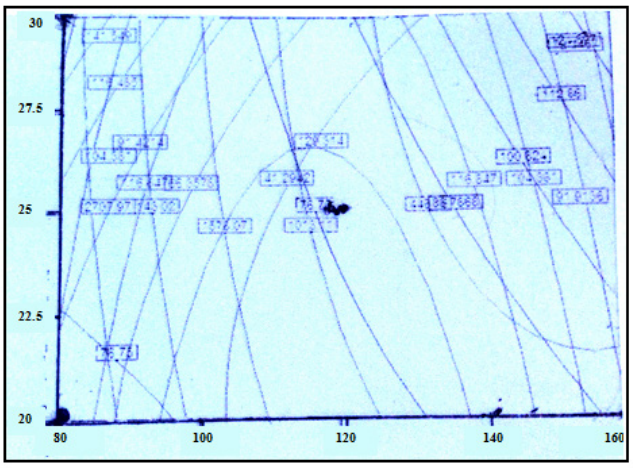


Figure 3 Superimposed contour plots of total phenolics, total monomeric anthocyanins, EC50 and total free sugar of black rice as a function of dry heating temperature and time.

ACKNOWLEDGEMENTS

This research was supported by Research Scholarships, Graduate School of Chulalongkorn University, Thailand.

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