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ORIGINAL ARTICLE

Thermal and pH degradation kinetics of anthocyanins in natural food colorant prepared from black rice bran

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Abstract The study of the stability of anthocyanins in food colorant powder is important to predict the quality changes occurring as the food products are processed, to prevent and control the degradation of the anthocyanins. The objectives of this study were to identify anthocyanin components in natural food colorants obtained from black rice bran, and investigate their thermal stability at 60, 80, and 100 °C, pH stability from 2.0 to 5.0 and also their correlation with visual color, L^*, C^*, and $h°$. Results showed that only six types of anthocyanins, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, delphinidin, cyanidin, pelargonidin and malvidin were present in raw black rice bran (BRB) and black rice bran colorant powder (BCP). The thermal degradation of both the visual color and the anthocyanin content in the BCP followed a first-order kinetic reaction model. The temperature-dependent degradation was adequately fitted to the Arrhenius equation. In terms of the pH stability, increasing pH values resulted in lower activation energies (E_a) and higher half-life ($t_{1/2}$) values for both color parameters and individual anthocyanins when heating from 60 to 100 °C. Moreover, the degradation rate constant (k) increased with increasing temperature and pH value. The degradation of cyanidin-3-*O*-glucoside and total anthocyanins showed a strong positive correlation with C^*. The changes in visual color may be used as an on-line quality control indicator during thermal processing of food products containing rice bran colorants which have high anthocyanin content.

Keywords Black rice bran · Anthocyanins · Degradation · Stability · Food colorants

Highlights
- Natural food colorant powder was prepared from black rice bran.
- Thermal and pH stability of the colorant powder were investigated.
- Correlations between anthocyanin degradation and visual color were investigated.
- Six types of anthocyanins were found in black rice bran and colorant powder.
- The degradation of total anthocyanins showed a strong positive correlation with C^*.

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Introduction

The use of natural anthocyanin pigments as coloring agents in food products is receiving increasing attention as they are attractive to consumers and have positive health benefits (Chou et al. 2007). Anthocyanin pigments are permitted as natural food colorants in the USA under the category of fruits (21 CFR 73.250) and vegetables (21 CFR 73.260), and the EU classification number is E163 (Lipman 1996; Wrolstad 2000). They are water-soluble glycosides and acylglycosides of the anthocyanidins of many fruits, vegetables and cereal grains. They are found in the form of polyhydroxylated and or methoxylated heterosides, which are derived from the flavylium ion or 2-phenylbenzopyrilium (Wu et al. 2004; Castañeda-Ovando et al. 2009). Currently, some 250 anthocyanins have been identified, however, only six of these, pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin, are commonly found in fruits and cereal grains (Escribano-Bailon et al. 2004). These compounds have been recognized as health-enhancing substances, owing to their antioxidant activity, anti-inflammatory properties and hypoglycaemic effects (Nam et al. 2006; Philpott et al. 2004). They also show other biological effects, including antimutagenic and anticarcinogenic activities (Hyun and Chung 2004).

Black rice (*Oryza sativa* L.) is becoming increasingly popular and is widely consumed in China, Japan, Korea and other East Asian countries such as Thailand (Pereira-Caro et al. 2013; Hou et al. 2013). Black rice bran (BRB), a waste product from the rice milling process, has gained recent attention for its potential use as a functional food because it contains high levels of polyphenols, especially anthocyanins, which are mainly found in the pericarp and aleurone layers of the bran fraction removed from the rice during the milling process (Jang and Xu 2009; Yawadio et al. 2007). Moreover, the bran is a rich source of other bioactive substances such as tocopherols, tocotrienols and γ-oryzanol (Loypimai et al. 2009; Ryynänen et al. 2004), which are well known as beneficial compounds for human health. However, the major current problem for the use of anthocyanins as food colorants has been limited by their relative instability under varying light, oxygen, temperature, enzymes, thermal treatment, co-pigment and pH conditions as well as other factors (Hou et al. 2013; Zhang et al. 2013). Preventing and controlling the degradation of anthocyanins in natural colorants is critical. In order to predict the quality changes occurring during food processing such as thermal processing and altering pH value, this study investigated the stability of the anthocyanins in food colorant powder prepared from black rice bran under several food processing conditions. Thermal degradation has been previously reported for BRB anthocyanins (Hou et al. 2013), blackberry juice and concentrate (Wang and Xu 2007), purple-fleshed sweet potato anthocyanins (Jie et al. 2013), and freeze-dried Roselle colorants (Duangmal et al. 2008). Visual color is an important physical and sensory property for product quality evaluation. The correlation between visual color and anthocyanin content during thermal processing in Urmu mulberry concentrate (Kara and Ercelebi 2013), blood orange juice (Shao-qian et al. 2011), and purple corn (*Zea mays* L.) (Yang et al. 2008) has been reported. This study was carried out to investigate the stability of the anthocyanins by focusing on the effect of temperature, time and pH on the degradation of individual the anthocyanins obtained from the BRB. The relationship between changes in visual color (L^*, C^*, and $h°$) and anthocyanin degradation during the thermal treatment of BRB colorant was examined. The findings will be useful in preparing appropriate food colorants and assist in establishing thermal and pH processing guidelines for food products. Visual color is an on-line quality parameter which can be used to predict anthocyanin degradation during thermal processing.

Materials and methods

Materials and chemicals

A BRB (*Oryza sativa* L.; waxy type) sample was obtained from a rice-milling factory (10 % degree of milling) in Roi-Et Province, Thailand. The raw bran from the milling process was immediately passed through a 20-mesh sieve to remove broken pieces of rice and husks. The moisture content was determined according to the method of AOAC (2000) (10.21 %).

Standards of cyanidin-3-*O*-glucoside chloride, dephinidin, pelargonidin, cyanidin, malvidin, cyanidin-3-*O*-rutinoside chloride, and maltodextrin (DE 4-7) were purchased from Sigma-Aldrich Chemical Co., (St. Louis, USA). High-performance liquid chromatography (HPLC) grade methanol, acetonitrile, hexane, acetic acid, ethyl acetate and ethanol were purchased from BDH Chemicals (Poole, UK). All chemicals and reagents were of analytical grade.

Preparation of black rice bran colorant powder (BCP)

The bran sample was added to deionised water to adjust the moisture content (MC) to 40 % (%wet basis) following optimal conditions and then placed in a chamber using ohmic heating, as reported by our previous study (Loypimai et al. 2015). Immediately after heating, the bran sample was removed from the chamber, cooled to room temperature, placed in a polyethylene bag and kept at 4 °C. The treated bran was extracted following the method reported by Duangmal et al. (2008) with some modifications. The 20 g sample from the ohmic treatment was extracted with 100 ml of acidified hydroalcoholic solution (water: 95 %, ethanol: 1:1, acidified with 0.1 M HCl to obtain a pH value of 2.5). The bran and solution were mixed using a mixer (Velp scientific, Europe) for 1 min before shaking in an orbital shaker (Gerhardt LS500, UK) at 100 rpm for 3 h. The slurry was filtered through a V-700 vacuum pump (Buchi, Switzerland) using Whatman No. 4 filter paper. The extract was added to maltodextrin (2 g/100 ml) and frozen at −50 °C before freeze-drying in a freeze dryer (FTS system Dura-Dry^{Tm}, USA) under a 27–33 Pa vacuum at a condenser temperature of −50 °C for 20 h. The dried sample was weighed, ground into a powder, and passed through a 50 mesh sieve. The colorant powder was kept in a brown glass bottle (45 ml) and placed in a desiccator for storage at 4 °C until required for analysis.

Qualitative and quantitative analysis using an HPLC-PDA (photodiode array detector)

The HPLC system consisted of a Shimadzu (Kyoto, Japan) LC-20AC series pumping system, a SPD-M20A diode array detector, and a SIL-10AD series auto-injector. The column was an Apollo C18 (Alltech Associates, Deerfield, IL, USA) (ø4.6 × 250 mm, 5 μm) protected with a Inertsil ODS-3 guard column (ø 4.0 × 10 mm, 5 μm; GL Science Inc., Tokyo, Japan). A 20 μl aliquot of each sample was used. The elution

conditions, described previously by Durst and Wrolstad (2001), were used with modifications. The mobile phase consisted of solvent A (acetonitrile, CH_3CN) and solvent B (4 % phosphoric acid, H_3PO_4) with the following gradient: 94 to 75 % B from 0 to 65 min, 75 to 94 % B from 65 to 70 min, and isocratic at 94 % B from 70 to 75 min to equilibrate the column for the next injection. Spectral data were recorded from 200 to 600 nm and the anthocyanin chromatograms were monitored at 520 nm. The operating conditions were column temperature 40 °C, injection volume 20 μl, detection at 520 nm, and flow rate of 1.0 ml/min. Quantification was performed by comparing the retention times and the spectra as well as by the addition of standards. The chromatograms were recorded and the peak areas were used to calculate the concentration of the anthocyanins against the calibration curve of the external standards.

Stability study

The kinetics of colorless and individual anthocyanins in the BCP were studied at 60, 80, 100 °C and at four different pH values (2.0, 3.0, 4.0, and 5.0). The BCP solution was prepared as previously described by Hou et al. (2013), with slight modifications. Three grams of BCP were dissolved in 1000 ml of 2.0 M acetate buffer. The pH values were adjusted to 2.0, 3.0, 4.0, and 5.0, respectively. Aliquots of 10 ml of each colorant solution were transferred to 15 ml brown glass vials and covered with a plastic cap to avoid evaporation of the thermally sensitive compounds. They were then placed in a thermoelectric water bath (PolyScience®, USA) and preheated to the desired temperature (60, 80, and 100 °C). For each temperature, 28 vials (seven vials for each pH) were randomly taken at 20 min intervals (0, 20, 40, 60, 80, 100, and 120 min) and cooled in an ice bath to stop thermal degradation. An aliquot of each sample was passed through a 0.45 μm nylon syringe filter (Whatman, USA) and injected into the HPLC system for analysis of the anthocyanin degradation.

The visual color of the solution sample was measured in terms of the CIELAB L^*, a^*, and b^* values using a Hunter Lab instrument (MiniScan, USA). L^* represents the lightness (L^* = 0 yields black and L^* = 100 indicates diffuse white). The Chroma (C^*) represents the color intensity, which is the distance of a color from the origin ($a^* = b^* = 0$) in the a^* and b^* plane. Hue angle ($h°$) is expressed in degrees from 0 to 360°, where 0° (red) is located on the $+a^*$ axis, and then rotates anticlockwise to 90° (yellow) for the $+b^*$ axis, 180° (green) for $-a^*$, and 270° (blue) for $-b^*$.

Previous studies (Jie et al. 2013; Hou et al. 2013; Yang et al. 2008), indicated the first-order reaction model for the degradation of most natural anthocyanin pigments from various sources. The first-order reaction rate constants (k in min^{-1}), the time needed for 50 % degradation in the visual color and anthocyanins, or half-life ($t_{1/2}$ in h) was calculated using the following equations:

$$C_t = C_0 \exp(k.t) \quad (1)$$

$$t_{1/2} = -\ln 0.5/k \quad (2)$$

Dependence of the degradation rate constant on temperature was determined by applying the Arrhenius equation:

$$K_T = K_0\, e^{\frac{-E_a}{RT}} \quad (3)$$

Where C_0 is the initial color parameter or anthocyanin content and C_t is the color value and anthocyanin content after t minutes of heating at a desired temperature. E_a is the activation energy ($kJ.mol^{-1}$), ln (k_T) is the kinetic constant at specified temperature, k_0 is a pre-exponential factor (min^{-1}), R is the universal gas constant (8.314 J/mol.K), and T is the absolute temperature (K). Activation energies were calculated by plotting ln (k_T) against the reciprocal of the absolute temperature ($1/T$), where the slope of the linear graph is equivalent to $\frac{-E_a}{RT}$.

Statistic analysis

The stability of the color parameters and the anthocyanins of the BCP were analyzed using a linear regression model to obtain the degradation rate constants (k) and activation energy (E_a). Analysis of variance (ANOVA) was performed using an SPSS program (trial version). Mean values were compared using Duncan's multiple range tests, and significant difference was defined at $P < 0.05$.

Results and discussion

Identification of anthocyanins

The anthocyanins are the major flavonoids in BRB. These compounds are responsible for the dark colors of black or purple, and are located mainly in the aleurone and pericarp layers of the rice bran. Anthocyanins and the visual color of the rice bran and BCP are listed in Table 1. The results indicate that only six types of anthocyanins, namely cyanidin-3-*O*-glucoside (Cy-3-glu), cyanidin-3-*O*-rutinoside (Cy-3-rut), delphinidin (De), cyanidin (Cy), pelargonidin (Pe), and malvidin (Mv), were detected. The major anthocyanin in the BRB and BCP was Cy-3-glu (62.5 % in BRB and 57.7 % in BCP), followed by De and Pe, respectively. In this study, the BCP showed a sevenfold increase in Cy-3-glu and a fourfold increase in the total content, compared with that of the BRB. Mv was only found in a small amount (51.56 μg/g in BRB and 101.8 μg/g in the BCP). Results also showed that the total anthocyanins (12,540.8 μg/g) in the BCP had a slightly higher

Table 1 Anthocyanin content and visual color of raw black rice bran (BRB) and black rice bran colorant powder (BCP)

Characteristics	BRB	BCP
Anthocyanins[a] (μg/g)		
Cyanidin-3-*O*-glucoside	1042.4 ± 10.5	7235.5 ± 18.3
Cyanidin-3-*O*-rutinoside	27.3 ± 3.19	93.25 ± 10.2
Delphinidin	451.3 ± 7.33	723.8 ± 19.7
Cyanidin	184.3 ± 8.35	638.4 ± 23.3
Pelargonidin	334.9 ± 10.2	1654.2 ± 54.2
Malvidin	51.6 ± 3.98	101.8 ± 11.2
Total contents	2947.3 ± 22.5	12,540.8 ± 85.9
Color value		
L^*	37.2 ± 0.84	39.4 ± 0.95
C^*	15.2 ± 0.38	17.2 ± 0.48
$h°$	310.9 ± 6.61	348.8 ± 6.74

[a] Values are means ±SD of triplicate samples (n = 3) (on a wet weight basis)

content (9480 μg/g) than those reported by Nontasan et al. (2012). According to the study of Jang and Xu (2009), Cy-3-glu is the predominate anthocyanin in the black rice. In contrast, lower concentrations, and some different anthocyanin profiles, were found in Japanese black–purple rice (Pereira-Caro et al. 2013), Chinese and Korean black rice powder (Hou et al. 2013; Frank et al. 2012), and American black rice (Zhang et al. 2013). The variation of anthocyanins may be attributed to different cultivars of pigmented rice, variable growing conditions, and the degree of rice milling.

The visual color measurements in terms of the L^*, $^*C$ and $h°$ values of the BCP were 39.41, 17.19, and 348.8, respectively. The BCP obtained was dark purple in color, and a darker shade than that of the BRB (Table 1). This finding is in agreement with the results documented by Mozetic et al. (2004), who reported that changes in C^* values strongly correlated to changes in anthocyanin content, and can be considered a good indicator of anthocyanin concentration. This may be attributed to the higher number of anthocyanins in the colorant form.

Anthocyanin stability

Thermal degradation of anthocyanins in the BCP was investigated at 60, 80, and 100 °C and under different acidity conditions (pH 2.0, 3.0, 4.0, and 5.0). Relative changes in individual anthocyanin content, with reference to the unconditioned sample (control) (C_t/C_0) whilst heating, were plotted against a regular time interval of 20 min (Fig. 1a). The kinetic parameters of degradation of the anthocyanins, including Cy-3-glu, Cy-3-rut, De, Pe, Cy, and Mv, and the total anthocyanin content while heating are displayed in Table 2. It was observed that the thermal degradation of individual anthocyanins and

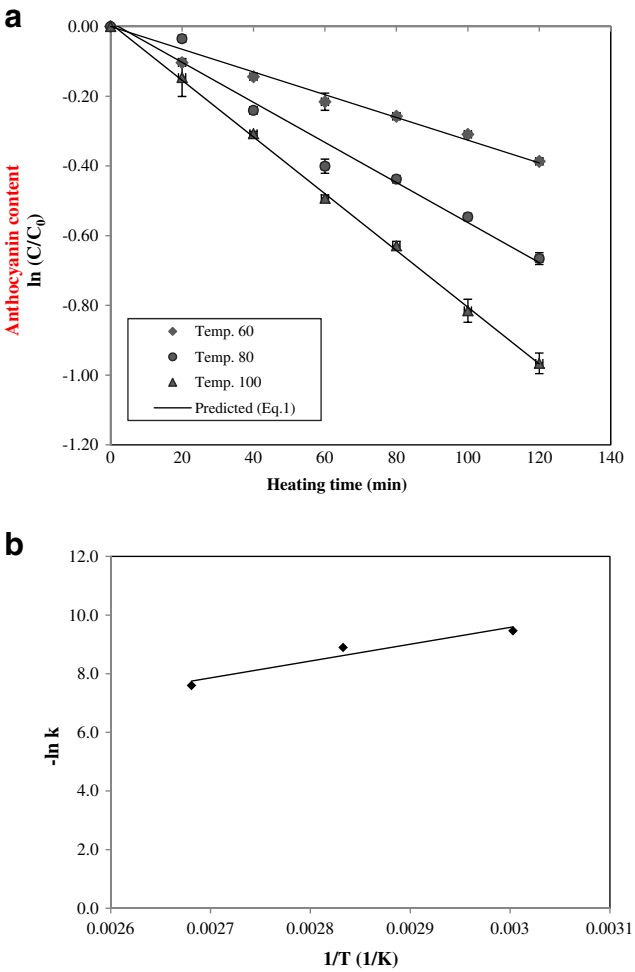

Fig. 1 Degradation of cyanidin-3-*O*-glucoside in black bran colorant during heating at 60, 80, and 100 °C, and at pH = 2.0 (**a**), and its Arrhenius pots for degradation at pH = 2.0 (**b**)

the total contents clearly followed the first-order reaction kinetic model with a high regression coefficient ($0.8647 < R^2 < 0.9887$). This result was in agreement with previous studies that reported the first-order reaction model for the degradation of the anthocyanin content in BRB (Hou et al. 2013), purple-fleshed sweet potato anthocyanins (Jie et al. 2013), blackberry juice and concentrate (Wang and Xu 2007) as well as freeze-dried Roselle colorants (Duangmal et al. 2008).

The kinetic rate constant (k) is an indicator that enables the prediction of the thermal degradation of anthocyanins. The lower the k value, the better the anthocyanin stability. The k values of individual anthocyanins and the total content significantly increased ($p < 0.05$) as the temperature increased from 60 to 100 °C. The highest k value was observed at 100 °C with a pH value of 5.0 ($p < 0.05$). The increase in the stability of anthocyanin pigment with a decline in the half-life value ($t_{1/2}$) was also less significant ($p < 0.05$). The greatest $t_{1/2}$ value of the colorant was observed in Cy-3-glu (26.9 h), followed by total anthocyanins (19.6 h) and Pe (15.9 h), respectively. This

Table 2 Influence of temperature and pH level on the kinetic rate constant (k) and half-life time ($t_{1/2}$) values of individual anthocyanins and total content degradation in black rice bran colorant powder (BCP)

pH	Temperature (°C)	Cy-3-glu		Cy-3-rut		De		Cy		Pe		Total contents	
		$k \times 10^3$ (min^{-1})	$t_{1/2}$ (h)	$k \times 10^3$ (min^{-1})	$t_{1/2}$ (h)	$k \times 10^3$ (min^{-1})	$t_{1/2}$ (h)	$k \times 10^3$ (min^{-1})	$t_{1/2}$ (h)	$k \times 10^3$ (min^{-1})	$t_{1/2}$ (h)	$k \times 10^3$ (min^{-1})	$t_{1/2}$ (h)
2.0	60	0.43^{a} $(0.9314)^{X}$	26.9^{a}	0.77^{a} (0.9356)	15.0^{a}	0.94^{a} (0.9578)	12.3^{a}	0.85^{a} (0.9639)	13.6^{a}	0.73^{a} (0.9729)	15.9^{a}	0.59^{a} (0.9284)	19.6^{a}
	80	0.52^{a} (0.8752)	22.1^{b}	0.99^{b} (0.9308)	11.6^{bc}	1.79^{b} (0.9692)	6.45^{b}	1.03^{a} (0.9225)	11.2^{b}	1.68^{b} (0.9707)	6.88^{b}	1.04^{b} (0.8795)	11.1^{c}
	100	1.16^{c} (0.9014)	9.96^{cd}	2.42^{e} (0.9524)	4.77^{de}	4.36^{d} (0.9355)	2.65^{d}	3.78^{f} (0.8954)	3.06^{f}	3.96^{e} (0.9568)	2.92^{d}	3.81^{e} (0.9552)	3.03^{f}
3.0	60	0.98^{b} (0.9653)	11.9^{c}	0.83^{ab} (0.9708)	13.9^{ab}	1.73^{b} (0.9015)	6.68^{b}	1.84^{b} (0.9005)	6.28^{c}	2.87^{c} (0.9028)	4.03^{c}	0.71^{a} (0.9629)	16.3^{b}
	80	1.19^{c} (0.9306)	9.71^{cd}	1.71^{d} (0.9528)	6.76^{d}	2.44^{c} (0.9245)	4.73^{c}	2.17^{c} (0.9125)	5.32^{cd}	3.46^{d} (0.9285)	3.34^{cd}	1.26^{c} (0.9066)	9.17^{d}
	100	1.85^{e} (0.9313)	6.24^{def}	3.61^{g} (0.8946)	3.20^{ef}	6.32^{g} (0.9039)	1.83^{def}	5.93^{h} (0.9228)	1.95^{g}	7.18^{g} (0.9271)	1.61^{e}	4.12^{f} (0.8863)	2.80^{g}
4.0	60	1.31^{cd} (0.9425)	8.82^{cd}	1.21^{c} (0.9528)	9.55^{c}	2.46^{c} (0.9244)	4.70^{c}	2.67^{d} (0.9139)	4.33^{de}	3.46^{d} (0.8647)	3.34^{cd}	2.16^{d} (0.9213)	5.35^{e}
	80	2.47^{f} (0.8944)	4.68^{efg}	2.82^{f} (0.9254)	4.10^{def}	4.75^{e} (0.9412)	2.43^{de}	3.12^{e} (0.9293)	3.70^{ef}	7.76^{h} (0.9002)	1.49^{f}	3.67^{e} (0.9509)	3.15^{f}
	100	3.16^{g} (0.9484)	3.66^{fg}	4.84^{h} (0.9246)	2.39^{ef}	9.71^{i} (0.9021)	1.19^{fg}	7.26^{i} (0.8994)	1.59^{gh}	9.45^{i} (0.9334)	1.22^{g}	4.87^{g} (0.8759)	2.37^{h}
5.0	60	1.41^{d} (0.9224)	8.19^{cde}	2.44^{e} (0.9301)	4.73^{de}	5.98^{f} (0.9215)	1.93^{def}	4.37^{g} (0.9025)	2.64^{fg}	6.73^{f} (0.9035)	1.72^{e}	5.34^{h} (0.9248)	2.16^{i}
	80	3.85^{h} (0.8848)	3.00^{fg}	5.18^{i} (0.9208)	2.23^{ef}	7.51^{h} (0.9435)	1.54^{ef}	5.87^{h} (0.8941)	1.97^{g}	10.4^{j} (0.9110)	1.11^{g}	8.59^{i} (0.9075)	1.34^{j}
	100	4.73^{i} (0.8966)	2.44^{g}	7.33^{j} (0.8842)	1.58^{f}	18.8^{j} (0.9184)	0.61^{g}	15.2^{j} (0.9212)	0.76^{h}	16.1^{k} (0.8868)	0.72^{h}	12.3^{j} (0.9887)	0.94^{k}
	% CV	6.85	7.67	8.97	4.52	9.11	7.31	4.68	4.59	6.71	8.10	8.23	3.68

Values are means of triplicate samples ($n = 3$)

Values with the same letters along the same columns are not significantly different ($p < 0.05$)

CV Coefficient of variation

Cy-3-glu cyanindin-3-*O*-glucoside

Cy-3-rut cyanindin-3-*O*-rutinoside

De delphinidin

Cy cyanidin

Pe pelargonidin

[X] correlation coefficient (R^2)

observation showed that Cy-3-glu was more stable than the other derivative anthocyanins for the black rice bran colorant. However, the $t_{1/2}$ values of Cy-3-glu and Cy-3-rut were higher than those reported by Hou et al. (2013). This may be attributed to the fact that the colorant powder prepared in this study contained a stabilizer (maltodextrin), which was used to encapsulate the pigment, resulting in enhanced anthocyanin stability. The combination of the flavylium cation form of the anthocyanins and dextrin retarded their transformation to other less-stable forms (Chandra et al. 1993). Moreover, the colorant powder with the maltodextrin addition may prevent a change in state from powder to a sorption gel. In this study, pH ranges could be another influencing factor on the anthocyanin degradation. Results are similar to those of Fleschhut et al. (2006), and Kennedy and Waterhouse (2000), showing that the anthocyanin pigment was found in different chemical forms, depending on the pH value of the solution. At a pH value of 1.0, the flavylium cation (red color) is the predominant form and contributes to the purple and red colors. At pH values between 2.0 and 4.0, the quinoidal blue species are predominant. At pH values between 5.0 and 6.0, only two colorless species can be detected, carbinol pseudobase and chalcone. Torskangerpoll and Andersen (2005) indicated that the pigment degradation of anthocyanin depended highly on the pH and anthocyanin structure. Hou et al. (2013) reported that the $t_{1/2}$ values of black rice anthocyanins diluted with citrate–phosphate buffer at pH 1.0 were greater than at pH 2.0–6.0. On the contrary, Cevallos-Casals and Cisneros-Zevallos (2004) reported that anthocyanins from the extracts of red sweet potato, purple corn, and commercial purple carrot colorants were more stable at pH 3.0 than at pH 1.0, at a temperature of 98 °C. In addition, the stability of the anthocyanin pigment is affected by several factors other than the pH value and heat treatment, such as storage temperature, chemical structure, concentration, light, oxygen, solvents, the presence of enzymes, flavonoids, proteins, and metallic ions (Rein 2005).

Degradation of visual color

The color change of BCP following the first-order reaction kinetics with the determined coefficient (R^2) greater than 0.8943 (Table 3) clearly showed that either pH range or heat treatment had a significant influence on the stability of the color values (L^*, C^*, and h^0) ($P < 0.05$). During heating, a change in C^* value with high k and $t_{1/2}$values was observed, whereas the $L^*$and h^0 values showed only small changes, as shown in Fig. 2. In addition, the k and $t_{1/2}$ values increased with an increase in temperature and pH value. The increasing temperature and time resulted in anthocyanin pigments of all samples becoming darker in all pH values, corresponding to significant decreases ($P < 0.05$) in L^* and C^* values as indicators for browning. This conclusion was supported by Lozano and Ibarz (1997) who reported that the change in L^* could be used to measure the browning of a heat-treated concentrated fruit pulp.

Table 3 Influence of temperature and pH level on the kinetic rate constant (k) and half-life time ($t_{1/2}$) values of visual color degradation in black rice bran colorant (BCP)

pH	Temperature (°C)	L^*		C^*		h^o	
		$k \times 10^3$ (min^{-1})	$t_{1/2}$ (h)	$k \times 10^3$ (min^{-1})	$t_{1/2}$ (h)	$k \times 10^3$ (min^{-1})	$t_{1/2}$ (h)
2.0	60	1.53^{a} (0.9669)^{X}	7.55^{a}	2.93^{a} (0.9749)	3.94^{a}	1.01^{a} (0.9359)	11.4^{a}
	80	3.27^{b} (0.9804)	3.53^{b}	5.55^{b} (0.9596)	2.08^{c}	1.51^{bc} (0.9729)	7.65^{bc}
	100	4.30^{c} (0.9497)	2.69^{c}	7.12^{c} (0.9888)	1.62^{d}	1.79^{c} (0.9795)	6.45^{cd}
3.0	60	3.01^{b} (0.9435)	3.84^{b}	3.57^{a} (0.9133)	3.24^{b}	1.39^{b} (0.9679)	8.31^{b}
	80	5.54^{d} (0.9189)	2.09^{d}	9.82^{e} (0.9864)	1.18^{de}	2.37^{de} (0.9766)	4.87^{ef}
	100	6.64^{e} (0.9201)	1.74^{e}	16.1^{h} (0.9675)	0.72^{gh}	3.74^{g} (0.9779)	3.09^{gh}
4.0	60	4.73^{c} (0.9088)	2.44^{c}	7.61^{c} (0.9250)	1.52^{d}	1.85^{c} (0.9729)	6.24^{cde}
	80	7.73^{f} (0.9817)	1.49^{f}	11.3^{f} (0.9125)	1.02^{efg}	2.20^{d} (0.9842)	5.25^{def}
	100	8.65^{g} (0.9629)	1.34^{g}	17.1^{i} (0.9218)	0.68^{gh}	2.38^{de} (0.9566)	4.85^{ef}
5.0	60	8.75^{gh} (0.9025)	1.32^{g}	8.34^{d} (0.9296)	1.39^{de}	2.60^{ef} (0.9396)	4.44^{fg}
	80	9.15^{gh} (0.9258)	1.26^{h}	15.2^{g} (0.9547)	0.76^{fgh}	2.81^{f} (0.9723)	4.11^{fg}
	100	9.28^{h} (0.8943)	1.24^{h}	24.8^{j} (0.9084)	0.47^{h}	4.43^{h} (0.9128)	2.61^{h}
	% CV	9.56	10.5	7.56	7.65	8.52	5.10

Values are means of triplicate samples ($n = 3$)

Values with the same letters along the same columns are not significantly different ($p < 0.05$)

CV Coefficient of variation

^{X} correlation coefficient (R^2)

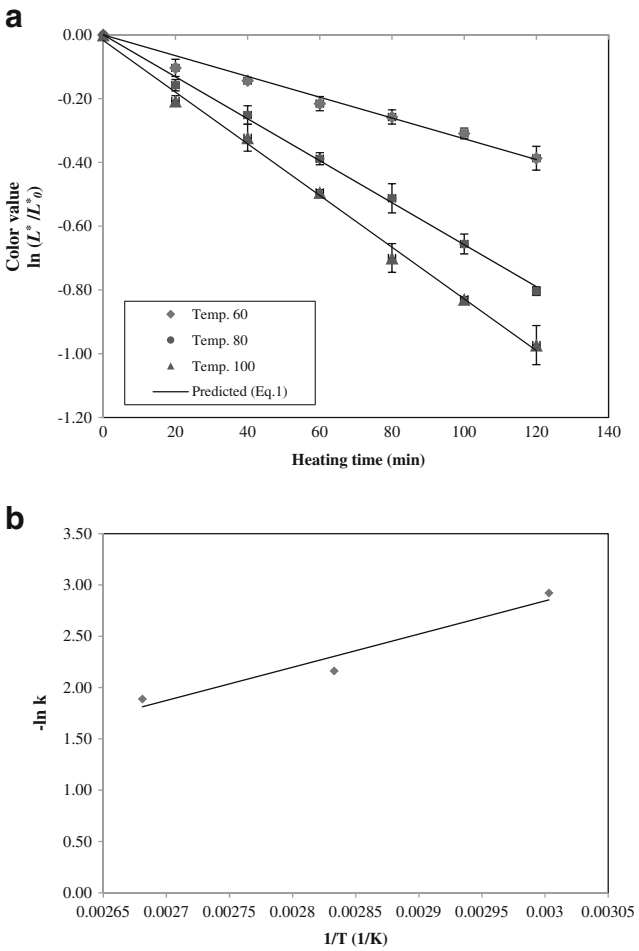

Fig. 2 Degradation of L^* value in black rice bran colorant solution during heating at 60, 80, and 100 °C, and at pH = 3.0 (**a**), and the Arrhenius plot for degradation at pH = 3.0 (**b**)

The dependence of color and anthocyanin degradation of BCP at temperatures of 60, 80, and 100 °C and various pH levels (range 2.0 to 5.0), and the effect on several parameters, were determined. The activation energy (E_a) was calculated by plotting $\ln(k_T)$ against the reciprocal of the absolute temperature ($1/T$), where the slope of the linear graph is equivalent to $\frac{-E_a}{RT}$ to fit the Arrhenius equation, (Eq. 3, Fig. 1b). The E_a of black rice bran colorant anthocyanins during heating was highest at pH 2.0; the values were 31.60 (Cy-3-glu), 37.87 (Cy-3-rut), 39.45 (De), 37.94 (Cy), 43.60 (Pe), and 46.75 (total contents) kJ mol^{-1}, respectively (Table 4.). The E_a of Pe and total contents were highest, which indicated that they were the most stable at low pH values. In addition, a high pH level was more sensitive to temperature changes, with high anthocyanin degradation. These results agreed with the findings of Hou et al. (2013). The anthocyanins in BRB, in a reaction with higher activation energy, were less stable, even though there were only small changes in temperature. In contrast, a higher stability of anthocyanins was obtained in aqueous solutions with pH 3.0 and 4.0 for apple and pear juices (Jie et al. 2013).

Regarding color change analysis, the $E_a$value for color, that is, for changes in L^*, C^*, and $h°$ at pH 2.0, was better than those at other pH values (Fig. 2b and Table 5). The C^* change had the highest value ofE_a. At higher pH values (4.0–5.0), the E_a values of color changes in L^*, C^*, and $h°$ ranged from 15.75 to 1.53 kJmol^{-1}, 23.09 to 20.87 kJmol^{-1}, and 13.56 to 6.55 kJmol^{-1}, respectively. From the E_a parameter studied, it can be determined that color changes and anthocyanin degradation are strongly correlated to the E_a value. They were more stable at lower temperatures with higher E_a values.

Relationship between visual color and anthocyanin content

During heating of black rice bran colorant solution at different pH values, the correlation between changes in the visual color

Table 4 Influence of pH level on the activation energy (E_a(kJ.mol^{-1})) of anthocyanin degradation in black rice bran colorant (BCP)

pH	Cy-3-glu	Cy-3-rut	De	Cy	Pe	Total content
2.0	31.60^{a} (0.8942)^{X}	37.87^{a} (0.9982)	39.45^{a} (0.9844)	37.94^{a} (0.9203)	43.60^{a} (0.9983)	47.75^{a} (0.9362)
3.0	25.36^{b} (0.8712)	35.90^{b} (0.9222)	35.38^{b} (0.9968)	31.83^{b} (0.8963)	26.21^{b} (0.9099)	45.05^{b} (0.9477)
4.0	22.90^{c} (0.9539)	29.19^{c} (0.8862)	33.12^{c} (0.9142)	29.77^{c} (0.9295)	23.38^{c} (0.8745)	21.09^{c} (0.9802)
5.0	16.40^{d} (0.9407)	28.57^{c} (0.9691)	29.20^{d} (0.8731)	25.46^{d} (0.9403)	22.49^{c} (0.9987)	17.54^{d} (0.9977)
% CV	5.91	9.68	5.12	7.50	8.65	4.41

Values are means of triplicate samples ($n = 3$)

Values with the same letters along the same columns are not significantly different ($p < 0.05$)

CV Coefficient of variation

Cy-3-glu cyanindin-3-*O*-glucoside

Cy-3-rut cyanindin-3-*O* -rutinoside

De delphinidin

Cy cyanidin

Pe pelargonidin

^{X} correlation coefficient (R^2)

Table 5 Influence pH level on the activation energy (E_a (kJ.mol^{-1})) of color degradation in black rice bran colorant (BCP)

pH	L^*	C^*	$h°$
2.0	26.89^a (0.9470)^X	39.10^a (0.9736)	25.56^a (0.9918)
3.0	20.61^b (0.9285)	28.16^b (0.9931)	14.87^b (0.9616)
4.0	15.75^c (0.9041)	23.09^c (0.9543)	13.56^b (0.8833)
5.0	1.53^d (0.9344)	20.87^d (0.9979)	6.55^c (0.9676)
% CV	6.58	5.52	8.57

Values are means of triplicate samples ($n = 3$)

Values with the same letters along the same columns are not significantly different ($p < 0.05$)

CV Coefficient of variation

^{X} correlation coefficient (R^2)

and the anthocyanin degradation was described using a linear relationship, as shown in Fig. 3.The graph shows the relationship between color values and Cy-3-glu concentration which is the major anthocyanin in black rice bran colorant. Degradation of each anthocyanin and total content was positively correlated with L^*, C^*, and $h°$ values. Interestingly, the C^* value showed high correlation with Cy-3-glu ($R^2 > 0.9342$) and total anthocyanins ($R^2 > 0.9211$). This excellent linear correlation inferred that the visual color parameters (L^*, C^*, and $h°$) may also be used instead of anthocyanins for black rice bran colorant. This might be due to the decrease in visual color especially C^*predominantly caused by the destruction of anthocyanin pigments upon heating, resulting in transformation of BCP solution from dark to other colorless forms. A similar result was recorded by Mozetic et al. (2004), who reported that changes in C^* strongly related to changes in anthocyanin content. Duangmal et al. (2008) found that the degradation of a drink with Roselle anthocyanin powder added was highly correlated to changes in L^* and C^*. A similar result of the changes in a^*, b^*, and L^* values was also reported by Kara and Ercelebi (2013) in Urmumulberry concentrate. This study was carried out on a model solution. However, the colorant powder with high anthocyanin content is possible to use in acidic foods and beverages. This has been confirmed by our study on the application of the colorant powder to prepare functional yogurt high in anthocyanin. Moreover, visual color in terms of C^* relating to the pigment concentration may be considered as an indicator for an on-line quality control to predict the anthocyanin degradation during thermal processing. Further studies are still needed to prove these suggestions.

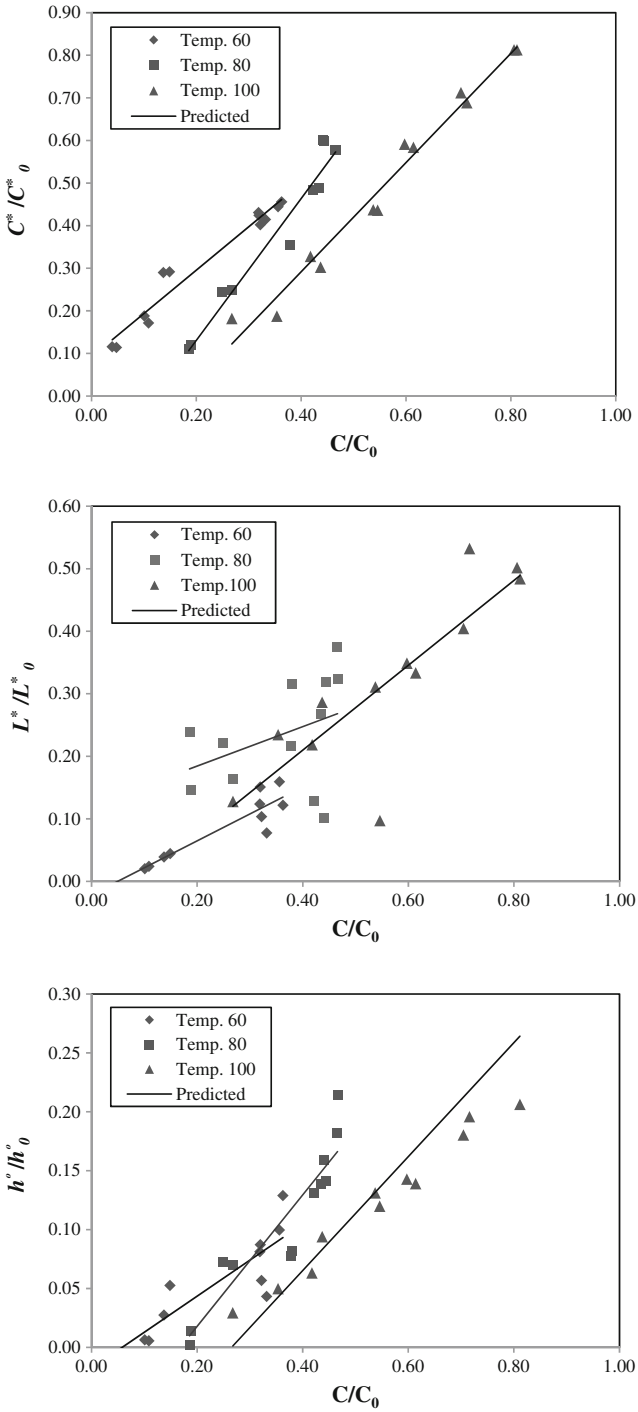

Fig. 3 Relationship between visual color (C^*, L^*, and $h°$ values) and anthocyanin content (Cy-3-glu) of black rice bran colorant solution during thermal treatment at pH 2.0

Conclusions

Only six types of anthocyanin, Cy-3-glu, Cy-3-rut, De, Cy, Pe, and Mv were present in the BRB and BCP. The major anthocyanin identified was Cy-3-glu. BCP had a sevenfold increase in Cy-3-glu and fourfold increase in total anthocyanin content compared to BRB.

This study indicated that thermal degradation of color and anthocyanins in BCP followed first-order reaction kinetics. The degradation of visual color and individual anthocyanin content also depended on temperature and pH level. A higher stability of color and anthocyanin pigments of BCP was

achieved at low temperature (60 °C) and a pH value of 2.0. The degradation of anthocyanins showed a strong positive correlation with C^* value. These findings are useful in establishing appropriate thermal processing guidelines and predicting anthocyanin degradation using visual color as an on-line quality control indicator.

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References

AOAC (2000) Official methods of analysis. Association of official analytical chemist international, 17th edn. AOAC International, Gaithersburg

Castañeda-Ovando A, Lourdes Pacheco-Hernández MDL, Páez-Hernández ME, Rodríguez JA, Galán-Vidal CA (2009) Chemical studies of anthocyanins: a review. Food Chem 113:859–871. doi:10.1016/j.foodchem.2008.09.001

Cevallos-Casals BA, Cisneros-Zevallos L (2004) Stability of anthocyanin-based aqueous extracts of Andean purple corn and redfleshed sweet potato compared to synthetic and natural colorants. Food Chem 86(1):69–77. doi:10.1016/j.foodchem.2003.08.011

Chandra A, Nair MG, Iezzoni AF (1993) Isolation and stabilization of anthocyanins from tart cherries (*Prunus cerasus* L.). J Agric Food Chem 41(7):1062–1065. doi:10.1021/jf00031a009

Chou PH, Matsui S, Misaki K, Matsuda T (2007) Isolation and identification of xenobiotic aryl hydrocarbon receptor ligands in dyeing wastewater. Environ Sci Technol 41(2):652–657. doi:10.1021/es061500g

Duangmal K, Saicheua B, Sueeprasan S (2008) Color evaluation of freeze-dried Roselle extract as a natural food colorant in a model system of a drink. J LWT Food Sci Technol 41:1437–1445. doi:10.1016/j.lwt.2007.08.014

Durst RW, Wrolstad RE (2001) In current protocols in food analytical chemistry. In: Wrolstad RE (ed), Wiley, New York, pp 1–13.

Escribano-Bailon MT, Santos-Buelga C, Rivas-Gonzalo JC (2004) Anthocyanins in cereals: Review. J Chromatogr A 1054:129–141

Fleschhut J, Kratzer F, Rechkemmer G, Kulling SE (2006) Stability and biotransformation of various dietary anthocyanins in vitro. Eur J Clin Nutr 45(1):7–18. doi:10.1007/s00394-005-0557-8

Frank T, Reichardt B, Shu Q, Engel HK (2012) Metabolite profiling of colored rice (*Oryza sativa* L.) grains. J Cereal Sci 55:112–119. doi:10.1016/j.jcs.2011.09.009

Hou Z, Qin P, Zhang Y, Cui S, Ren G (2013) Identification of anthocyanins isolated from black rice (*Oryza sativa* L.) and their degradation kinetics. Food Res Int 50(2):691–697. doi:10.1016/j.foodres.2011.07.037

Hyun JW, Chung HS (2004) Cyanidin and malvidin from *Oryza sativa cv.* Heungjinjubyeo mediate cytotoxicity against human monocytic leukemia cells by arrest of G(2)/M phase and induction of apoptosis. J Agric Food Chem 52:2213–2217. doi:10.1021/jf030370h

Jang S, Xu Z (2009) Lipophilic and hydrophilic antioxidants and their antioxidant activities in purple rice bran. J Agric Food Chem 57:858–862. doi:10.1021/jf803113c

Jie L, Xiao-ding L, Yun Z, Zheng-dong Z, Zhi-ya Q, Meng L, Shao-hua Z, Shuo L, Meng W, Lu Q (2013) Identification and thermal stability of purple-fleshed sweet potato anthocyanins in aqueous solutions with various pH values and fruit juices. Food Chem 136:1429–1434. doi:10.1016/j.foomchem.2012.09.054

Kara S, Ercelebi AE (2013) Thermal degradation kinetics of anthocyanins and visual colour of Urmu mulberry (*Morus nigra* L.). J Food Eng 116:541–547. doi:10.1016/j.jfoodeng.2012.12.030

Kennedy JA, Waterhouse AL (2000) Analysis of pigmented high-molecular mass grape phenolics using ion-pair, normal-phase high-performance liquid chromatography. J Chromatogr A 866(1):25–34

Lipman AL (1996) Current regulations for certification exempt color activities in the USA. In *Proceeding of the second international symposium on natural colorants INF/COL II.* Acapulco, Mexico: The Hereld Oraganization, S.I.C. Publishing.

Loypimai P, Moonggarm A, Chottanom P (2009) Effects of ohmic heating on lipase activity, bioactive compounds and antioxidant activity of rice bran. Aust J Basic Appl Sci 3(4):3642–3652

Loypimai P, Moonggarm A, Chottano P, Moontree T (2015) Ohmic heating-assisted extraction of anthocyanins from black rice bran to prepare a natural food colorant. Innov Food Sci Emerg Technol 27:102–110. doi:10.1016/j.ifset.2014.12.009

Lozano JE, Ibarz A (1997) Colour changes in concentrated fruit pulp during heating at high temperatures. J Food Eng 31:365–373

Mozetic B, Trebse P, Simcic M, Hribar J (2004) Changes of anthocyanins and hydroxycinnamic acids affecting the skin colour during maturation of sweet cherries (*Prunus avium* L.). LWT Food Sci Technol 37:123–128. doi:10.1016/S0023-6438(03)00143-9

Nam SH, Choi SP, Kang MY, Koh JH, Kozukue N, Friedman M (2006) Antioxidative activities of bran extracts from twenty one pigmented rice cultivars. Food Chem 94:613–620. doi:10.1016/j.foodchem.2004.12.010

Nontasan S, Moongngarm A, Deeseenthum S (2012) Application of functional colorant prepared from black rice bran in yogurt. APCBEE Procedia 2:62–67. doi:10.1016/j.apcbee.2012.06.012

Pereira-Caro G, Watanabe S, Crozier A, Fujimura T, Yokota T (2013) Phytochemical profile of a Japanese black-purple rice. Food Chem 141:2821–2827. doi:10.1016/j.foodchem.2013.05.100

Philpott M, Gould KS, Lim C, Ferguson L (2004) In situ and in vitro antioxidant activity of sweet potato anthocyanins. J Agric Food Chem 52(6):1511–15113. doi:10.1021/jf034593j

Rein M (2005) Copigmentation reactions and color stability of berry anthocyanins. University of Helsinki, Helsinki, pp. 10–14

Ryynänen M, Lampi A, Salo-Väänänen P, Ollilainen V, Piironen VA (2004) A small-scale sample preparation method with HPLC analysis for determination of tocopherols and tocotrienols in cereals. J Food Compos Anal 17:749–765. doi:10.1016/j.jfca.2003.09.014

Shao-qian C, Liang L, Si-yi P (2011) Thermal degradation kinetics of anthocyanins and visual color of blood orange juice. Agric Sci China 10(12):1992–1997. doi:10.1016/S1671-2927(11)60201-0

Torskangerpoll K, Andersen OM (2005) Colour stability of anthocyanins in aqueous solutions at various pH values. Food Chem 89(3):427–440. doi:10.1016/j.foodchem.2004.03.002

Wang WD, Xu SY (2007) Degradation kinetics of anthocyanins in blackberry juice and concentrate. J Food Eng 82(3):271–275. doi:10.1016/j.jfoodeng.2007.01.018

Wrolstad RE (2000) Anthocyaninis. In: Lauro GJ, Francis FJ (eds) Natural food colorants: science and technology. Marcel Dekker, New York

Wu X, Gu L, Prior RL, McKay S (2004) Characterization of anthocyanins and proanthocyanidins in some cultivars of ribes, aronia, and sambucus and their antioxidant capacity. J Agric Food Chem 52(26):7846–7856. doi:10.1021/jf0486850

Yang ZD, Han YB, Gu ZX, Fan GJ, Chen ZG (2008) Thermal degradation kinetics of aqueous anthocyanins and visual color of purple corn (*Zea may* L.) cob. Innov Food Sci Emerg Technol 9(3):341–347. doi:10.1016/j.ifset.2007.09.001

Yawadio R, Tanimori S, Morita N (2007) Identification of phenolic componds isolated from pigmented rices and their aldose reductase inhibitory activities. Food Chem 101(4):1616–1625. doi:10.1016/j.foodchem.2006.04.016

Zhang X, Shen Y, Prinyawiwatkul W, King JM, Xu Z (2013) Comparison of the activities of hydrophilic anthocyanins and lipophilic tocols in black rice bran against lipid oxidation. Food Chem 141:111–116. doi:10.1016/j.foodchem.2013.03.034