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# Thermal Stability of the Major Color Component, Cyanidin 3-glucoside, from a Korean Pigmented Rice Variety in Aqueous Solution

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**Abstract** : Thermal stability of the major color component, cyanidin 3-glucoside, isolated from Korean pigmented rice (*Oryza sativa* var. Suwon 415) were investigated to explore possible application of value-added natural colors as food additives. The anthocyanin showed red and blue color with maximum absorption peaks at 511 nm and 572 nm in acidic (pH 2.0) and alkaline (pH 9.0) buffer solutions, respectively, and the thermal degradation reactions were carried out with different temperature ranges at 50~90℃. Degree of degradation was determined with UV/Vis spectra which indicate characteristic absorption patterns with sharp isosbestic points at 350 nm (pH 2.0), and 275, 310, and 405 nm (pH 9.0). Thus the reaction follows simple first-order kinetics. The anthocyanin was very stable against heat at acidic pH and relatively stable at alkaline pH with half-life values of 50.3 hr and 0.6 hr at 70℃, respectively. The activation energies and Arrhenius frequency factors of the pigment were 26.9 kcal $mol^{-1}$ and $6.0\times10^{11}$ $s^{-1}$, at pH 2.0, and 15.2 kcal $mol^{-1}$ and $1.4\times10^{6}$ $s^{-1}$, at pH 9.0, respectively.(Received May 20, 1996; accepted June 13, 1996)

## Introduction

Anthocyanins are probably the most widespread water-soluble pigments in plant kingdom. Apparently harmless to health, they have been suggested as possible food colorants.[1,2] However, unlike synthetic dyes, anthocyanins have not been widely used as food colorants, because of their instability during storage and processing. The color and structure of anthocyanins sharply changes at different pH conditions,[5,6] and they were reported as relatively stable against heat at acidic conditions.[3,4] Thermal degradation reaction of anthocyanins follows first-order kinetics.[3] The reaction is also affected by pH, oxygen and light.[3,4,7,8]

Pigmented rice and also known as black rice is a red or purple colored rice and important food crops in the South Asia and China. Color components of pigmented rice are mainly anthocyanins[9,10] and especially, cyanidin 3-glucoside is the major anthocyanin pigment isolated from Japan[11] and Korea.[12,13] Purple pigments of pigmented rice were used as food colorants in the processing of bread, ice cream and beverages because of its stability against heat and light, and its high contents in rice hulls.[11,13] The anthocyanin pigments also possess antioxidative activity.[14]

In the previous study,[12,13] the major anthocyanin from Korean pigmented rice (*Oryza sativa* var. Suwon 415) was purified and identified as cyanidin 3-*O*-*β*-*D*-glucopyranoside. In this research, we determined the thermal stability of cyanidin 3-glucoside from Korean pigmented rice in acidic and alkaline buffer solutions to explore possible application of value-added natural food colors.

## Materials and Methods

### Materials

Pigmented rice (*Oryza sativa* var. Suwon 415) was kindly gifted from Dr. Moon, H.-P., National Crop Experiment Station, Rural Development Administration, Suwon, Korea, and stored at room temperature. Chromatography papers were purchased from Whatman Ltd. Amberlite XAD-7 and reagents for buffer preparation including phosphoric acid, sodium phosphate, CHES (2-[N-cyclohexylamino]-ethanesulfonic acid), citric acid, sodium citrate and glycine were purchased from Sigma Chemical Co. Other chemicals including methanol, n-butanol and pyridine were bought from Hayman Ltd.

### Purification of the major anthocyanin

The color component of pigmented rice was extracted

Key words : anthocyanin, pigmented rice, cyanidin 3-glucoside, half-life, activation energy
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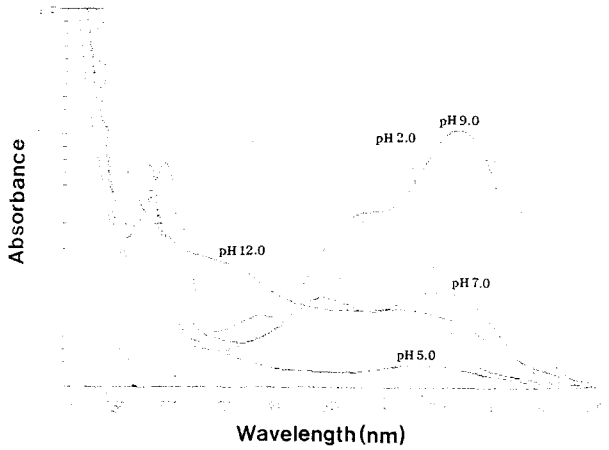


Fig. 1. UV/Vis spectra of cyanidin 3-glucoside from Korean pigmented rice cultivar at different pHs.

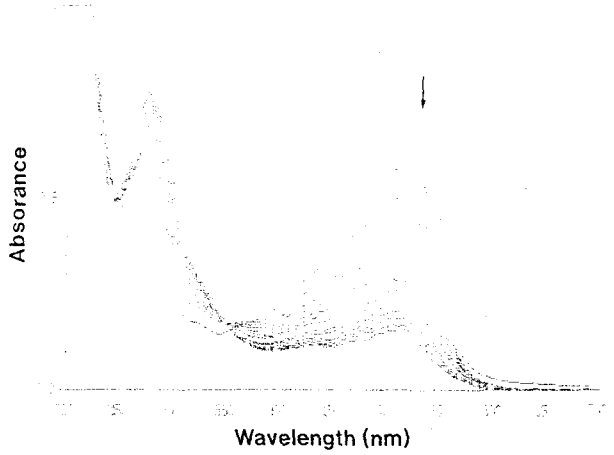


Fig. 2. UV/Vis specrum changes of the thermal degradation reactions of cyanidin 3-glucoside in 20 mM phosphate buffer, pH 2.0 at 90°C. Specra were scanned with 1h interval.

with 0.1% HCl-MeOH. The extract was concentrated to a small volume and applyed to Amberlite XAD-7 column. The pigment was eluted with $H_2O$-MeOH (4 : 1) and the elute was concentrated to a small volume. The concentrate was purified by preparative paper chromatography with BAW (n-BuOH : AcOH : $H_2O$=4 : 1 : 5). The major bands were cut and extracted with 0.01% HCl-MeOH and the extract was concentrated to a small volume. The purified anthocyanin, cyanidin 3-glucoside, was stored at −70℃ and used for further studies.

### UV/Vis spectrum changes of the purified anthocyanin at different pHs

UV/Vis spectrum changes of the purified anthocyanin were measured at different pHs using various buffer systems. Used buffer systems were 20 mM phosphate (pH 2.0, pH 7.0, pH 12.0), 20 mM citrate (pH 5.0) and 20 mM glycine (pH 9.0) buffers. UV/Vis spectra of the purified anthocyanin at various pHs were measured at the range of 200 −700 nm.

### Thermal degradation reactions

Thermal degradation reactions were carried out in acidic (pH 2.0) and alkaline (pH 9.0) buffer solutions. 20 mM phosphate buffer and 20 mM CHES buffer were used for pH 2.0 and pH 9.0, respectively. The anthocyanin samples dissolved in buffer solution were incubated in temperature-controlled UV/Vis spectrophotometer at different temperature ranges 50~90℃. Degree of degradation was measured by absorbance changes at 511 nm (pH 2.0) and 572 nm (pH 9.0) with UV/Vis spectrophotometer.

## Results and Discussion

### UV/Vis spectral changes of the purified cyanidin 3-glucoside at varying pH

The structures and spectra of anthocyanins are changed in aqueous solutions at varying pH.[5,6] The purified cyanidin 3-glucoside from Korea pigmented rice showed typical UV/Vis spectra at different pH values (Fig. 1), indicating that the anthocyanin possesses various structures at different pHs. In the case of pelargonidin 3-glucoside, structures are flavylium ion in acidic solution (pH<3), quinoidal base in slightly acidic solution (pH 4~6) and pseudobase in solution pH higher than 7.0.[5] The color of the purified cyanidin 3-glucoside was red at pH 2.0, colorless at pH 5.0, pale blue at pH 7.0, blue at pH 9.0 and pale yellow at pH 12.0. Because the anthocyanin showed intensive red and blue colors at pH 2.0 and pH 9.0, respectively, these conditions were chosen for the measurements of thermal degradation reactions.

### Effect of temperatures on color stability at acidic (pH 2.0) and alkaline (pH 9.0) conditions

The factors to affect color stability of anthocyanins are heat, pH, oxygen, metals and light.[3,4,7,8] Among these factors, heat is the most important factor to affect color stability. Thermal degradation reaction of the purified anthocyanin was carried out in acidic (pH 2.0, 20 mM phosphate) and alkaline (pH 9.0, 20 mM CHES) buffer solutions at different temperature ranges 50~90℃. When the anthocyanin in acidic buffer was incubated at 90℃, red ($\lambda_{max}$=511 nm) color was gradually disappeared, yielding colorless solution with sharp isosbestic point at 350 nm (Fig. 2). The cyanidin 3-glucoside was also gradually degraded in alkaline buffer at 60℃, yielding pale yellow color with isosbestic point at 275, 310, and 405 nm (Fig. 3). These results suggested that each of cyanidin 3-glucoside at acidic and alkaline pH is degraded

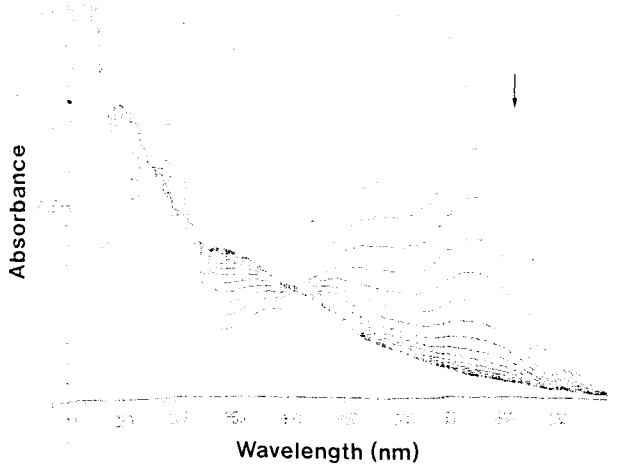


Fig. 3. UV/Vis specrum changes of the thermal degradation reactions in 20 mM CHES buffer, pH 9.0 at 60°C. Specra were scanned with 30 min interval.

Table 1. Rate constants($k$) and half-life($T_{1/2}$) of thermal degradation reactions of cyanidin 3-glucoside at acidic (pH 2.0) and alkaline (pH 9.0) pHs at different temperatures.

| Temperature (℃) | Rate constant ($s^{-1}$) | | Half-life (h) | |
|---|---|---|---|---|
| | pH 2.0 | pH 9.0 | pH 2.0 | pH 9.0 |
| 50 | | $7.7\times10^{-5}$ | | 2.5 |
| 60 | | $1.5\times10^{-4}$ | | 1.3 |
| 70 | $3.8\times10^{-6}$ | $3.2\times10^{-4}$ | 50 | 0.61 |
| 75 | $7.8\times10^{-6}$ | | 25 | |
| 80 | $1.3\times10^{-5}$ | $5.8\times10^{-4}$ | 15 | 0.33 |
| 85 | $2.0\times10^{-5}$ | | 9.7 | |
| 90 | $3.6\times10^{-5}$ | $1.0\times10^{-3}$ | 5.3 | 0.19 |

to produce single degradation product. Degree of degradation was determined by measuring absorbance changes at 511 nm (pH 2.0) and 572 nm (pH 9.0). When degree of degradation was plotted on a semilogarithmic scale, the plot showed straight lines at each pH. Thus the reaction follows simple first-order kinetics. It was reported that thermal degradation reactions of betanine and cranberry anthocyanins follow first-order kinetics and is also affected by pH and light.[3] The rate constants and half-life values of the reaction were summarized in Table 1. The thermal degradation rates at pH 2.0 were slower two orders of magnitude than those at pH 9.0 indicating that cyanidin 3-glucoside was much more stable at acidic pH than alkaline pH. The half-life values of cyanidin 3-arabinoside and cyanidin 3-galactoside from cranberry at 55℃ in acidic condition (pH 2.5) are 41h and 43h in the absence of light, respectively.[3] The thermal degradation rate constants of sunflower hull anthocyanin pigments at pH 3.0 were $6.1\times10^{-6}s^{-1}$ and $3.4\times10^{-5}s^{-1}$ at 65℃ and 80℃, respectively.[4] These values are comparable with present data even though purity of the sample

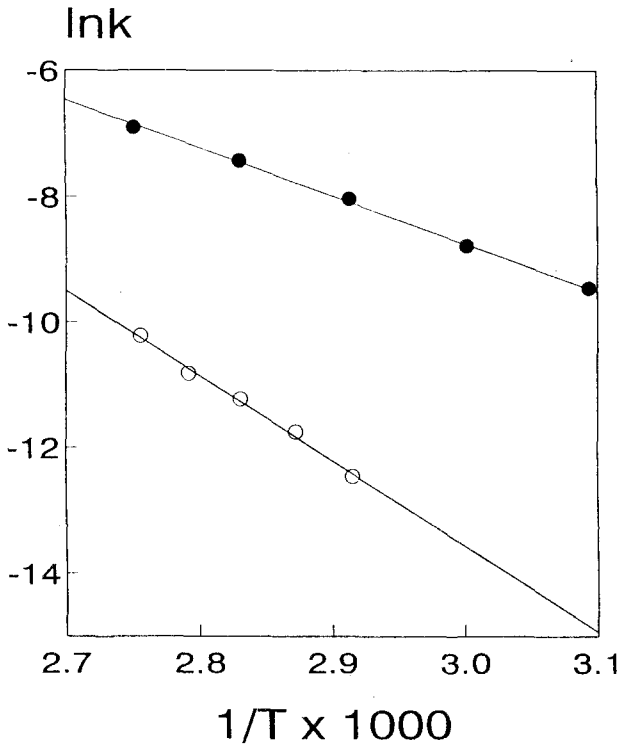


Fig. 4. Arrhnius plots of the thermal degradation reactions of cyanidin 3-glucoside at pH 2.0 (○—○) and pH 9.0 (●—●).

and reaction conditions were different each other. Arrhenius plots of thermal degradation reactions were shown in Fig. 4. The activation energies ($E_a$) and Arrhenius frequency factors (A) of the reactions were 26.9 kcal $mol^{-1}$ and $6.0\times10^{11}$ $s^{-1}$, at pH 2.0, and 15.2 kcal $mol^{-1}$ and $1.4\times10^{6}$ $s^{-1}$, at pH 9.0, respectively. The activation energies of cyanidin 3-arabinoside and cyanidin 3-galactoside from cranberry at pH 2.5 were 26.8 kcal $mol^{-1}$ and 26.7 kcal $mol^{-1}$ in the absence of light, respectively,[3] suggesting that the activation energies of thermal degradation of cyanidin anthocyanins are essentially identical regardless of sugar moieties.

In summary, the cyanidin 3-glucoside isolated from Korean pigmented black rice showed intense red and blue color with maximum absorption peaks at 511 nm and 572 nm in acidic (pH 2.0) and alkaline (pH 9.0) buffer solutions, respectively. The pigment was much more stable to heat at acidic (pH 2.0) condition than at alkaline (pH 9.0) condition. The rate constants and activation energies of the thermal degradation of cyanidin 3-glucoside were comparable with other cyanidin glycosides studied thus far.

## Acknowledgement

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**한국산 유색미의 주요성분인 Cyanidin 3-glucoside의 수용액에서의 열안정성**

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**초록** : 식품첨가물로서의 활용가능성을 검색하기위해, 한국산 유색미(*Oryza sativa* var. Suwon 415)의 주요색소인 cyanidin 3-glucoside의 열안정성과 pH 변화에 따른 UV/Vis 스펙트럼의 변화에 대해 연구하였다. 유색미의 안토시아닌 색소는 산성(pH 2.0)과 염기성(pH 9.0) 완충용액에서 각각 강한 적색과 청색을 나타내었고 열에 의한 색소의 분해반응은 20 mM phosphate (pH 2.0)와 20 mM CHES (pH 9.0) 완충용액에서 50~90℃의 온도범위에서 수행되었다. 색소의 분해정도는 UV/Vis 스펙트럼의 변화를 측정하여 결정하였으며, 350 nm (pH 2.0)와, 275, 310, 및 405 nm (pH 9.0)에서 isosbestic point를 나타내었다. 이 결과는 색소분해반응이 1차 반응임을 의미한다. 유색미 안토시아닌은 산성조건에서 매우 안정하였고 염기성조건에서도 비교적 안정하였으며, 반감기는 70℃에서 각각 50.3 h과 0.6 h이었다. 색소분해반응의 활성화에너지($E_a$)와 Arrhenius frequence factor(A)는 pH 2.0에서는 26.9 kcal $mol^{-1}$과 $6.0\times10^{11}$ $s^{-1}$이었고, pH 9.0에서는 15.2 kcal $mol^{-1}$과 $1.4\times10^{6}$ $s^{-1}$이었다.

*연락저자