KOREAN J. FOOD SCI. TECHNOL.
Vol. 21, No. 3, pp 446~452 (1989)

Red Pigment of the Korean Cockcomb Flower: Color Stability of the Red Pigment

S. Y. Lee, S. J. Cho*, K. A. Lee, P. H. Byun**and S. M. Byun

Department of Biological Science and Engineering, Korea Advanced Institute of Science and Technology

**Department of Home Economics Education, Seo Won University*

***Department of Food and Nutrition, Dong-Duck Women's University*

한국산 맨드래미 꽃의 적색 색소 : 적색 색소의 식품학적 안정성

이상열·조숙자*·이경애·변평화**·변시명

한국과학기술원 생물공학과

*서원대학교 가정교육과, **동덕여자대학교 식품영양학과

Abstract

The pigment of the Korean cockscomb flower, a betacyanin, was evaluated for its stability in terms of temperature, pH, and its behavior upon exposure to water, light, and air. The pigment was the most stable at pH 4.0, and its activation energy (*Ea*) for degradation was shown to be 17.55 Kcal/mol. In general, sugars protected against color degradation at the concentration of 0.1M. Degradation of this pigment in the presence of food constituents, such as organic acids, metal ions, or antioxidants, at the concentrations normally present in food preparations, can be kept to a minimum by selective adjustment of conditions. This pigment, therefore, has potential value as a food colorant under selected conditions.

Keywords: natural red pigment, betacyanin

Introduction

Because synthetic food colorants have come under scrutiny by regulatory agencies as a result of potential health hazards, it has become necessary to explore natural pigments as potential food colorants. For use as a natural red pigment, beet concentrate is commercially obtained from red beet roots; the pigment content of beet concentrate is 0.1-1.0%. Adams *et al.*[1] obtained a pigment content of 6-8% in the final product by fermentation of beet juice with *Candida utilis*. As an alternative, Weller and Lausure[2] experimented with beet tissue cultures either on agar or in suspension. Betacyanin, being a natural pigment, can be safely used in food products[3] including sausage[4] yogurt, ice cream and sherbet[5] and maraschino cherries[6].

The chemical and physical properties of red beet pigment have been well characterized in terms of heat and pH stability[7,8], effects of water activity[5], effect of light and air[9,10], effects of sterilization[11], and their stability in food products[4] and in solutions[12-15].

Korean cockscomb flowers contain an average of 3-5% betacyanin in their dried flowers; this betacyanin is very similar to red beet betacyanin[16]. The flower has long been used for coloring certain traditional Korean foods, and it is widely available. It is a horticultural plant which produces large red flowers in the shape of an inverted triangle.

To evaluate this flower pigment as a potential alternative to red pigment derived from beets, this study was performed in order to characterize its stability when exposed to various conditions similar to the of food preparation.

Corresponding author: Si Myung Byun, Department of Biological Science and Engineering, KAIST. POB 150 Chungryang, Seoul

Materials and Methods

Pigment preparation

Fresh Korean cockscomb flowers were harvested in autumn from about 50 trees representing major red strains of several species widely grown in Korea. The flowers were dried in a dark place and then ground fine enough to pass through an 80-mesh screen. Twenty grams of the powder were extracted three times with 200 m*l* of H_2O and were then filtered through a 0.45μm MF Millipore filter under reduced pressure. Six grams of crude pigment were obtained after the combined filtrates were lyophilized. This crude pigment preparation was used for most studies, unless otherwise specified. In certain instances, flower betacyanin purified by 1.2% preparative agarose gel electrophoresis, as described elsewhere[16], was used. Authentic red beet pigment was provided by Meer Co. (U.S.A.) in the form of a concentrate.

Temperature effect

The degradation rate of the red flower pigment was determined according to the method of von Elbe *et al.*,[7] by measuring the decrease in light adsorption at the maximum absorption wavelength, because of the simplicity of this assay. One mg of crude pigment was dissolved in 10 m*l* of sterilized H_2O (pH 7.0), and 1 m*l* of this solution was sealed tightly in a 5-m*l* vial after repeated flushing with nitrogen gas to limit oxygen. Samples were heated at various temperatures for 5, 10, 20, 30, 60, and 90 min. The absorbances at 537 nm were measured before and after heat treatment by appropriate dilutions. The percent residual was calculated. For thermal kinetic data, the purified flower pigment was used instead. All determinations were performed in triplicate.

pH effects

The effect of pH on the stability of the red pigment was determined according to the methods of von Elbe *et al.*[7] and Saguy[8], with a slight modification. One mg of the crude pigment was dissolved in 10 m*l* of various pH solutions prepared with 0.1N NaOH and 0.1H HCl. One m*l* of each solution was introduced into a 5-m*l* vial, which was flushed repeatedly with nitrogen gas and sealed tightly. Vials were maintained at 4 °C in the dark and absorbances at 537 Nm were measured initially and at regular intervals for one month. All determinations were performed in triplicate.

Effects of light and/or air

One m*l* of the crude pigment solution, prepared as described in "Temperature effect" was transfered into a 5-m*l* vial, which was flushed several times with nitrogen gas and sealed tightly. Samples to be exposed to light were stored in daylight. The samples without exposure to light were stored in vials wrapped in aluminum foil. Similar experiments were carried out to determine the effect of air. Samples to be exposed to air were not flushed with nitrogen gas, and were stored in the dark without sealing. Samples without exposure to air were stored in vials which were flushed with nitrogen gas and sealed. All samples were stored at room temperature, and all determinations were performed in triplicate.

Water activity (Aw) effect

One mg of the crude pigment was dissolved in 10 m*l* of various *Aw* solutions which were prepared by adjusting the ratio of water: glycerol according to the method of Pasch and von Elbe[17]. One aliquot of each (1 m*l*) was transfered into a 5-m*l* vial and incubated at 70 °C in a water bath. The residual pigment was determined by measuring absorbances at 537 nm.

Effects of sugars, metal ions, organic acids, and antioxidants

Selected sugars (glucose, fructose, galactose, maltose, sucrose, lactose), metal ions (Fe^{++}, Cu^{++}, Hg^{++}, Mn^{++}, Mg^{++}, Co^{++}, Pb^{++}, Cd^{++}, Al^{+++}, K^{+}), organic acids (citric, malic, succinic, lactic, fumaric, acetic, oxalic), and antioxidants (ascorbic, thiourea, α-tocopherol, sodium pyro-

phosphate) were examined for their effect on degradation of red pigment from the cockscomb flower. Concentrations of sugar, metal ion, organic acid, and antioxidant used were 0.1M, 10 and 100 pm, 1 mEq, and up to 3,000 ppm, respectively. The species and their concentrations were chosen because they are common food constituents or food contaminants. The samples, prepared as described under "Temperature effect", were mixed with various compounds and stored at 4 ℃ in the dark. Absorbance changes at 537 nm were measured. All determinations were performed in triplicate.

Results and Discussion

Thermal degradation

Fig. 1 shows the thermal degradation rate of the red flower pigment, illustrating first-order degradation kinetics. The slope of the lines indicates that the degradation rate can be expressed in term of the rate constant, k. From these data, the activation energy (Ea) was calculated from a semi-logarithmic plot of k *vs* 1/T and was found to be 17.55 Kcal/mol.

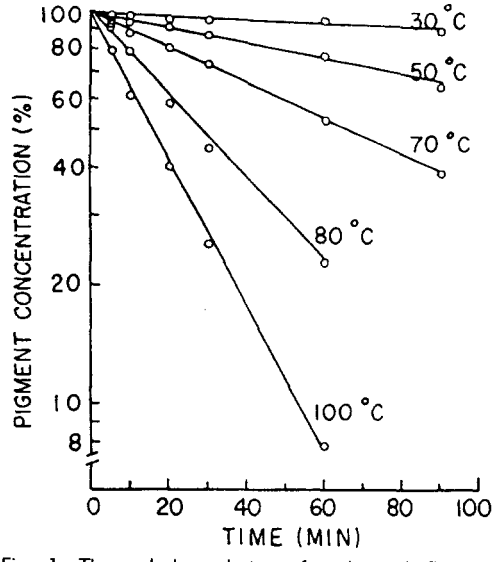

Fig. 1. Thermal degradation of cockscomb flower red pigment as a function of time and temperature at pH 7.0.

When red beet betacyanin was heated for various time periods, the red color gradually faded to a brownish color. Because of this degradation in color, determination of betacyanin by measuring absorbance at a single wavelength at 537 nm may lead to erroneous results[18-20]. In this study, however, the degree of pigment degradation was measured by following the decrease in light absorption at the maximjm wavelength. The method is simple and adequate for purposes of comparison, since the absorption at 430 nm, is negligible.

These data clearly indicate that the heat stability of red pigment from the cockscomb flower decreases with an increase in temperature. As reported in red beet betacyanin[7,8,14,21], the red flower pigment solution was also unstable at higher temperatures, especially above 50 °C.

The exact mechanism of degradation of the flower betacyanin under these experimental conditions is unknown. However, evidence suggests that the flower betacyanin degrades as follows: betanin ⟶ betalamic acid ⟶ betalamic acid cleavage product ⟶ browning substances[14]. We tested this with the purified red pigment of the cockscomb flower as well as with red beet betacyanin. As shown in Fig. 2, the absorption maximum at 537 nm gradually disappeared, and one at 430 nm (betalamic acid) appeared instead and then eventually faded out when the solution was heated

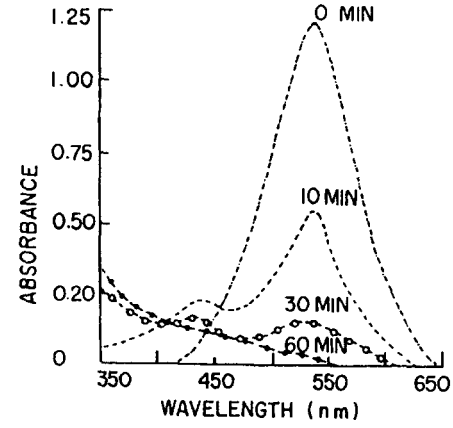

Fig. 2. Visible spectra of the degradation of cockscomb flower red pigment at 80 °C.

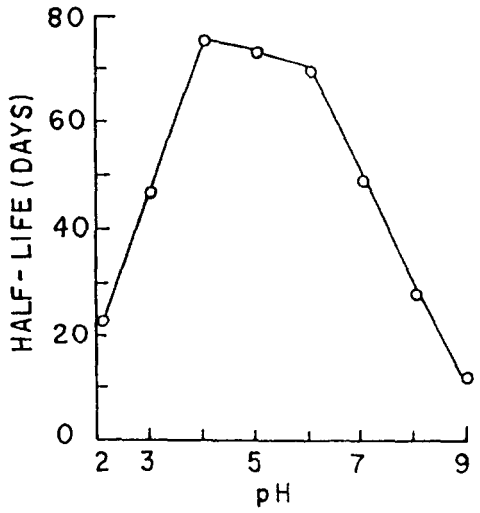

Fig. 3. Half-life ($T_{1/2}$) as a function of pH for cockscomb flower red pigment at 5 °C.

at 80 °C.

Effect of pH

Visible spectra of the red flower pigment at different pHs were identical to those of red beet betacyanin[16], showing absorption maxima of 539, 537, and 535 nm at pH 9, 5, and 2 for both betacyanins, respectively. These alterations were accompanied by a marked color change from red to violet. As shown in Fig. 3, pH effect on pigment stability was also expressed as $T_{1/2}$ according to different pHs at 4 °C. Maximum stability of the red flower pigment was observed at pH 4.0, while that of red beet pigment was observed at pH 5.0-5.8[8] or at pH 5.0-6.0[7]. Although special attention is required when the data are compared with each other, since substantial differences in pH stability can be brought about by variations in such factors as *Aw*, atmosphere, light, organic acids, metals, etc, the red flower pigment appeared to possess slightly better pH stability than did red beet pigment at more acidic conditions. This appeared to be due to the fact that the former contained organic acid components (ferulic acid, *p*-coumaric acid, and an unknown)[16]. Both pigments, from the red flower and the red beet, were the most stable at pH 4.0-6.0.

Table 1. Residual percentage of red pigment of Korean cockscomb flower after positive or negative exposure to light and air at room temperature for one day.

Condition	% Residual
− Light − Air	66.4
− Light + Air	59.0
+ Light − Air	56.5
+ Light + Air	48.7

The concentration of red pigment at 0 time was considered as 100%. (+), presence; (−), absence.

Effects of light and air

Since deleterious effects of oxygen or light on red beet betacyanin have been observed[7,10,11], sensitivity of the red flower pigment to degradation by light or air was determined by storing the pigment solution at pH 5.0. The presence of light increased the degradation rate by 10.0 ± 0.5%, the presence of air by 7.5 ± 0.5%, and the presence of both light and air by 17.5 ± 0.5% for the red flower pigment, as compared with 15.6 ± 0.5%, 14.6 ± 0.5%, and 28.6 ± 0.5% for red beet betacyanin, respectively[7], when it was stored at 15 °C for 6 days. The results are presented in Table 1.

Water activity (Aw) effect

Triplicate experiments were performed at each water: glycerol concentration and samples were incubated at 70 °C. The mean amount of residual pigment was determined and a plot of $T_{1/2}$ *vs* *Aw* was prepared, as shown in Fig. 4. Compared with the results with red beet betacyanin, the flower pigment had a relatively shorter $T_{1/2}$ at lower *Aw* and a longer $T_{1/2}$ at higher Aw. However, the $T_{1/2}$ of the flower pigment was nearly two times greater at *Aw* 0.37 than at Aw 1.0.

Effect of various constituents

Sugars are very common food components. The effects of various sugars on stability of the red flower pigment were evaluated in the presence of a 0.1M concentration of a variety of su-

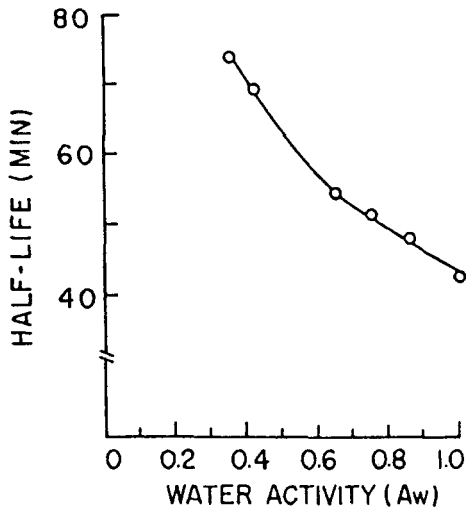

Fig. 4. Half-life ($T_{1/2}$) as a function of water activity (Aw) at 70°C.

gars. Table 2 shows the results. All of the sugars tested revealed that addition of sugar had protective effects against color degradation, as compared with control. The protective effect was also observed when the pigment was stored, even at 30°C for 24 h (data not shown).

Table 2. Effects of sugar (0.1M) on retention (residual percentage) of cockscomb flower red pigment at 4°C

Sugar	Residual %					Half-life (days)
	0 day	7th day	14th day	21th day	31th day	
Control	100	88.6	84.8	82.1	79.4	73.5
Glucose	100	89.2	89.6	88.1	82.7	92.3
Fructose	100	88.9	87.8	86.9	82.2	109.7
Galactose	100	89.4	87.6	87.1	84.1	109.9
Maltose	100	89.5	88.5	87.7	81.8	97.8
Sucrose	100	89.0	88.2	88.0	82.0	109.6
Lactose	100	88.5	87.9	87.2	81.9	106.5

Effects of metals on the red flower pigment were evaluated by addition of 10 and 100 ppm of various metal ions. As shown in Table 3, all of the metal ions, especially Cu^{++} and Hg^{++}, significantly decreased pigment stability. Also, the higher the concentration of metal ions added, the greater

Table 3. Effects of metal ions on retention (residual percentage) of the cockscomb flower red pigment at 4°C

Metal ion	Concentration (ppm)	Residual %					Half-life (days)
		0 day	7th day	14th day	21st day	31st day	
Control	0	100	88.6	84.8	82.1	79.4	73.5
Fe^{++}	10	100	83.7	80.8	73.8	60.1	42.0
	100	100	80.3	73.2	62.5	50.0	31.0
Cu^{++}	10	100	85.5	80.6	72.5	57.4	38.7
	100	100	62.3	59.0	53.3	43.2	23.2
Hg^{++}	10	100	77.4	64.3	55.5	43.5	24.7
	100	100	72.9	59.3	49.2	37.9	19.4
Mn^{++}	10	100	95.1	84.0	71.5	57.6	43.3
	100	100	90.1	78.5	65.2	50.3	31.3
Mg^{++}	10	100	90.0	87.1	76.0	52.0	45.8
	100	100	89.7	86.6	75.1	50.1	33.9
Co^{++}	10	100	83.2	83.1	74.6	59.2	40.9
	100	100	76.8	75.2	69.5	57.7	34.2
Pb^{++}	10	100	85.7	75.0	70.2	63.1	46.6
	100	100	85.4	73.4	65.3	43.7	30.5
Cd^{++}	10	100	82.8	78.7	69.0	61.2	39.3
	100	100	71.8	68.5	63.8	55.0	32.4
Al^{+++}	10	100	85.2	83.5	82.0	80.8	73.6
	100	100	80.9	77.0	74.5	73.3	49.4
K^{+}	10	100	85.4	79.5	78.0	74.5	58.5
	100	100	85.2	78.6	76.1	71.2	40.1

was the reduction of pigment stability. Pasch and von Elbe[15] explained this by the fact that the metal ions can act as electron donors or acceptors and can therby destabilize the electrophilic center, resulting in rearrangement of associated bonds and destruction of chromophore.

Since there are many organic acids in natural food systems, these effects on degradation of the red-flower pigment were also investigated, as presented in Table 4. In general, organic acids reduced pigment stability. However, large individual differences between the effects of different organic acids were not observed.

Degradation of the red pigment was much affected by oxygen, as shown in Table 1. Therefore, an antioxidant was added to the pigment solutions to test their protective effect against pigment degradation. However, as shown in Table 5, the antioxidant did not protect against degradation. This result is somewhat different from results achieved with red beet betacyanin. Pasch and von Elbe[15] reported that addition of 100 ppm α-tocopherol to the red beet betacyanin solution increased $T_{1/2}$ from 48.0 ± 1.0 min to 50.2 ± 3.4 min at pH 5.0, 75 °C. This protective effect was not detected for the red flower pigment after addition of α-tocopherol.

Table 4. Effects of organic acid (1 mEq) on retention (residual percentage) of the cockscomb flower red pigment at 4 °C

Organic acid	Residual %					Half-life (days)
	0 day	7th day	14th day	21th day	31th day	
Control	100	88.6	84.8	82.1	79.4	73.5
Citric	100	86.5	73.0	61.0	46.4	27.8
Malic	100	90.0	80.0	70.2	55.1	43.4
Succinic	100	90.3	81.0	71.0	56.4	42.4
Lactic	100	97.5	77.0	69.5	40.4	23.7
Fumaric	100	95.0	81.0	67.3	51.9	39.7
Formic	100	89.0	75.7	65.5	52.2	31.8
Acetic	100	90.8	81.5	72.5	59.9	50.2
Oxalic	100	92.5	85.4	76.0	60.8	42.0

In summary, although data for the addition of the various constituents discussed above were not in exact correlation between red pigments from the cockscomb flower and the red beet pigment, the general results were very similar. In conclusion, the effects of addition of these various constituents can be held to a minumum by selective adjustment of conditions for use of the red flower pigment in food preparations. The study of this subject was completed using model food systems and sensory studies regarding this red pigment were presented[22].

요 약

한국산 맨드래미 꽃의 적색 색소인 베타시아닌 색소의 식품학적 안정성을 조사하기 위하여 온도, pH, water activity, 빛 및 공기조절에 따른 변화를 관찰하였다. 이 적색 색소는 pH 4.0에서 가장 안정하였으

Table 5. Effects of antioxidant on retention (residual percentage) of the cockscomb flower red pigment at 4 °C

Antioxidant	Concentration (ppm)	Residual %					Half-life (days)
		0 day	7th day	14th day	21st day	31st day	
Control	0	100	88.6	84.8	82.1	79.4	73.5
Ascorbic acid	100	100	88.4	80.7	70.0	52.0	39.1
	1000	100	85.2	76.4	69.6	51.6	32.5
Thiourea	1000	100	79.9	75.0	64.8	44.6	26.6
	3000	100	79.0	75.0	62.5	42.2	24.9
α-Tocopherol	100	100	88.5	82.3	74.2	58.8	39.7
	1000	100	85.4	79.6	69.6	52.9	33.7
Sodium pyrophosphate	1000	100	82.5	78.3	69.3	51.1	32.0
	3000	100	80.0	75.7	66.3	48.3	29.5

며, 열에 대한 분해 활성화에너지 (*Ea*)는 17.55 kcal/mol 이었다. 일반적으로 당화합물의 첨가는 0.1M 농도에서 색소 분해 방지효과가 있었고, 식품속에 존재하는 농도의 유기산, 금속이온 및 항산화제들은 적색 색소의 분해를 심하게 일으키지 않음으로써, 이 적색 색소는 천연식품 색소로서 이용될 가능성이 증명되었다.

References

1. Adams, J.P., von Elbe, J.H. and Amundson, C.H.: Production of a betacyanin concentration by fermentation of red beed juice with *Candida utilis*. *J. Food Sci.* **42**, 78(1978)
2. Weller, T.A. and Lausure, L.L.: Betalains in beet tissue culture. *J. Food Sci.* **47**, 162(1981)
3. von Elbe, J.H. and Maing, I.Y.: Betaines as possible food colorants of meat substitutes. *Cereal Sci. Today* **18**(9), 263(1973)
4. von Elbe, J.H., Klement, J.T., Amundson, C.H., Cassens, R.G. and Lindsay, R.C.: Evaluation of betalaine pigments as sausage colorants. *J. Food Sci.* **39**, 128(1974)
5. Pasch, J.H., von Elbe, J.H. and Sell, R.J.: Betalains as colorants in dairy products. *J. Milk Food Technol.* **38**(1), 25(1975)
6. Anonymous; Bright red color substituted for red #4. *Food Proc. Apr.* **79**, 54(1979)
7. von Elbe, J.H., Maing, I.Y. and Amundson, C.H.: Color stability of betanin. *J. Food Sci.* **44**, 334(1974)
8. Saguy, I.: Thermostability of red beet pigments (betaine and vulgaxanthin-1): Influence of pH and temperature. *J. Food Sci.* **44**, 1554(1979)
9. Wiley, R.C. and Lee, Y.N.: Recovery of betalaines from red beets by a diffusion-extraction procedure. *J. Food Sci.* **43**, 1056(1978)
10. Wiley, R.C., Lee, Y.N., Saladini, J.J., Wyss, R.C. and Topalian, H.H.: Efficiency studies of a continuous diffusion apparatus for the recovery of betalaines from the red table beet. *J. Food Sci.* **44**, 208(1979)
11. Habib, A.T. and Brown, H.D.: The effect of oxygen and hydrogen ion concentration on color changes in processed beets, strawberries and rapsberries. *Proc. Am. Soc. Hort. Sci.* **68**, 482(1956)
12. Kopelman, I.J. and Saguy, I.: Color stability of beet powder. *J. Food Proc. Preser.* **1**, 217(1977)
13. Peterson, R.G. and Joslyn, M.A.: The red pigment of the root of the beet (*Beet vulgaris*) as a pyrole compound. *Food Res.* **25**, 429(1960)
14. Saguy, I., Kopelman, I.J. and Mizrahi, S.: Thermal kinetic degradation of betanine and betalamic acid. *J. Agri. Food Chem.* **26**(2), 360(1978)
15. Pasch, J.H. and von Elbe, J.H.: Betanine stability in buffered solution containing organic acids, metal cations, antioxidants, or sequestrants. *J. Food Sci.* **44**, 72(1979)
16. Lee, S.Y., Lee, K.A., Woo, S.J., Jo, J.S. and Byun, S.M.: Characterization of a red pigment of the Korean cockscomb flower. Biotechnol. and Appl. Biochem. to be submitted (1989)
17. Pasch, J.H. and von Elbe, J.H.: Betaine degradation as influenced by water activity. *J. Food Sci.* **40**, 1145(1975)
18. Saguy, I., Kopelman, I.J. and Mizrahi, S.: Computer-aided determination of beet pigments. *J. Food Sci.* **43**, 124(1978)
19. Schwartz, S.J., Hildenbrand, B.E. and von Elbe, J.H.: Comparison of spectrophotometric and HPLC methods to quantify betacyanins. *J. Food Sci.* **46**, 276(1981)
20. Attoe, E.L. and von Elbe, J.H.: Degradation kinetics of betanine in solutions as influenced by oxygen. *J. Agric. Food Chem.* **80**, 708(1982)
21. von Elbe, J.H.: Stability of betalaines as food colors. *Food Technol.* **29**, 42, May (1975)
22. Lee, S.Y., Shin, Y.C., Byun, S.M., Jo, J.S. and Cho, S.J.: Evaluation of red pigment of cockscomb flower in model food systems as a natural food colorant. *Korean J. of Food Science and Technology*, **18**(5), 388(1986)

(1989년 4월 4일 접수)