

## Effects of pH on the Coloration and Degradation of the Anthocyanins from the Root Tuber of *Panax notoginseng* (Burk) F. H. Chen

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**Abstract:** The effects of pH on the coloration and degradation of the root tuber anthocyanins of *Panax notoginseng* were studied *in vitro* by using spectrophotometry. The results indicated that both the visible light absorption spectra and the degradation rates of the anthocyanins were particularly pH-dependent. At pH 2.0, the anthocyanins expressed the strongest red. Along with the increase of pH values from 0 to 13.0, the maximal absorption wavelengths in visible light ( $\lambda_{\text{vis max}}$ s) of the anthocyanins expressed bathochromic and hypsochromic shift orderly, then disappeared, and the absorbance values at the maximal absorption wavelength in visible light ( $A\lambda_{\text{vis max}}$ s) exhibited a single-peak curve, with the peak at pH 2.0. After the primitive pH values were restored to 2.0, if the primitive pH  $\leq 6.0$ , the red hues of the anthocyanins were all resumed to much stronger, the  $\lambda_{\text{vis max}}$ s altered toward 532 nm in different degrees, and the  $A\lambda_{\text{vis max}}$ s increased, if the primitive pH  $\geq 7.0$ , the red hues of the anthocyanins could not be resumed at all, the  $\lambda_{\text{vis max}}$ s did not almost change, and the  $A\lambda_{\text{vis max}}$ s still remained at a very low level. Being placed at 15 °C in darkness, the anthocyanins at the pH values of 0 ~ 6.0 all degraded along with time. The degradation rate at pH 2.0 was the slowest, and when the pH  $\leq 3.0$ , the anthocyanins degraded slowly as a whole. Furthermore, the degradation courses of the anthocyanins almost approximated first-order reaction kinetics. This paper could provide a reference for the exploration on the mechanism of the root tuber coloration and the exploitation and utilization of the tuber pigments of *P. notoginseng*.

**Key words:** *Panax notoginseng* (Burk) F. H. Chen; root tuber; anthocyanin; pH; coloration; degradation; effect

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## pH 对三七块根花色苷颜色呈现和降解的效应\*

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**摘要:** 用分光光度法在体外研究了 pH 对三七块根花色苷呈色和降解的效应, 结果表明: 该花色苷在可见光区

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的吸收光谱和降解速率均具有独特的 pH 依赖性。在 pH 2.0, 该花色苷呈现最强烈的红色。随着 pH 从 0 增加到 13.0, 该花色苷在可见光区的最大吸收波长 ( $\lambda_{\text{vis max}}$ ) 依次出现红移、蓝移, 然后消失, 在可见光区最大吸收波长处的吸光值 ( $A_{\lambda_{\text{vis max}}}$ ) 呈现为一条单峰曲线, 唯一的峰在 pH 2.0 处。当原始 pH 值被恢复到 2.0 后, 如果原始 pH 值  $\leq 6.0$ , 花色苷的红色均被恢复得更浓烈,  $\lambda_{\text{vis max}}$  不同程度地趋向 532 nm,  $A_{\lambda_{\text{vis max}}}$  增加; 如果原始 pH 值  $\geq 7.0$ , 花色苷的红色根本不能被恢复,  $\lambda_{\text{vis max}}$  几乎不变,  $A_{\lambda_{\text{vis max}}}$  仍然维持低水平。在 15 °C, 黑暗中, 该花色苷在 pH 0 ~ 6.0 条件下均随时间而降解, 在 pH 2.0 时的降解速度最慢, 当 pH  $\leq 3.0$  时, 该花色苷在总体上降解缓慢; 此外, 该花色苷的降解过程几乎符合一级反应动力学。本文可为三七块根颜色呈现的机理探索及其色素的开发、利用提供参考。

**关键词:** 三七; 块根; 花色苷; pH; 颜色呈现; 降解; 效应

*Panax notoginseng* (Burk) F. H. Chen is one of the rare Chinese medicinal materials. It is usually named “the supernatural herb of South China”, and is the main ingredient of the world-famous “Yunnan Baiyao”. *P. notoginseng* has been proved to hold many pharmacological activities. It has the remedial functions of different degrees to the diseases of the cordis and cerebral vascular, neural and immune systems, etc, and the superexcellent activities of anti-inflammation, anti-senescence and anti-tumor<sup>[1-3]</sup>.

Although *P. notoginseng* distributes mainly in the regions with the altitudes from 1 200 to 2 000 m which are located between Yunnan and Guangxi provinces of China, and in the neighborhood of the Tropic of Cancer, the most concentrative planting region of *P. notoginseng* is Wenshan Eparchy of Yunnan province. Both the annual output and the quality of the root tuber of *P. notoginseng* in this eparchy are the best in China, resulting in the reality that *P. notoginseng* is “the first medicinal material” in Yunnan, *P. notoginseng* produced in the Wenshan is the genuine medicinal material of *P. notoginseng*<sup>[4]</sup>, and Wenshan Eparchy is “the village of *P. notoginseng* of China”. Now, the base-constructing of *P. notoginseng* in Wenshan Eparchy is carried out according to the standard operation procedure (SOP) of GAP<sup>[5,6]</sup>.

The root tuber is the primary medicinal part of *P. notoginseng*, and it is usually yellow or yellowish white. However, about 5% ~ 10% of root tubers were found to be purple first by the farmers in Wenshan, then by our research group. The purple tuber was observed to hold a plentiful of purplish red substance<sup>[7]</sup>. As such, the tubers were primarily classi-

fied into two categories, namely the green and the purple<sup>[7,8]</sup>. We have proved that the purple pigments of the root tuber belong to anthocyanins and, in effect, the root tubers of different colors all contain anthocyanins of different quantities<sup>[9]</sup>.

For many years, ginsenosides have been thought to be the most important pharmacological component of *P. notoginseng*<sup>[10,11]</sup>. But the existence of the purple root tuber of *P. notoginseng* means that the purple pigments, namely the anthocyanins, are also the pharmacological component of *P. notoginseng* which can not be ignored in any case. This is because anthocyanins has been proved to be provided with various medicinal and nutritional values for human being, such as antioxidation, scavenging the free radicals, adjusting the fat content in serum and liver, antimutagenicity, ameliorating microcirculation, improving eyesight, deferring agglomeration of hematoblast and preventing various diseases, e. g. cancer, cardiovascular diseases, arthritis and diabetes<sup>[12,13]</sup>. So, it is fully possible that the root tuber anthocyanins of *P. notoginseng per se* may hold special medicinal activities. Certainly, a careful characterization of the concrete activities of the anthocyanins is still waiting to be carried out.

Considering the potential importance of the root tuber anthocyanins of *P. notoginseng*, in this paper, we systemically reported the concrete effects of pH on the coloration and degradation of the tuber anthocyanins for the first time, which should provide a reference for the exploration on the mechanism of the root tuber coloration and the exploitation and utilization of the root tuber pigments.

## 1 Materials and methods

### 1.1 General

All solvents used were of analytical grade made in China. All reactions were carried out in capped test tubes and repeated two times. Visible light absorption spectrum was measured at 22 °C in a 1 cm pathlength quartz cell in the 400 ~ 700 nm range using a Shimadzu-2450 UV-visible spectrophotometer. After the solutions with pH values were made up, the pH values of the solutions were all verified by a Shimadzu-pH meter.

### 1.2 Plant material

40 root tubers of *P. notoginseng* were randomly selected and collected from Huazhuang Village of Matang Town of Wenshan Eparchy on Oct 14 of 2005. The hypsography of the fields selected is comparatively planus, the average altitude is 1580 m, and the soil belongs to loamy clay. Every root tuber was quickly cleaned with tap water, the exterior water of the tubers was absorbed entirely by filter papers at once, and then frozen at -20 ~ -22 °C.

### 1.3 Isolation of the anthocyanins from the root tuber of *P. notoginseng*

The frozen tubers were elementarily defrosted by a momentary wash under the tap water of about 15 °C. The exterior water of the tubers was absorbed entirely by filter papers. After being placed at 15 °C for 20 min, the tubers were cut up to the cubes of about 2 mm × 2 mm × 2 mm. 50.0 g of the cubes was ground quickly and completely at about 22 °C after mixing with approximate 50 mL methanol containing 1% concentrated HCl (V/V)<sup>[9,14]</sup>. Extracts were filtered and the residues were washed till they became full yellowish white. The final extract was diluted to 250 mL with the above acidic methanol, and refrigerated under 3 °C in darkness.

### 1.4 Design of the solutions with pH gradients ranging from 0.0 to 13.0

Solutions with pH gradients ranging from 0.0 to 13.0 were prepared by hydrochloric acid and four kinds of buffers. pH 0.0 was created by 1.00 mol/L

hydrochloric acid, pH 1.0 by 0.10 mol/L hydrochloric acid, pH 2.0 by 0.01 mol/L hydrochloric acid<sup>[15]</sup>. pH 3.0 ~ 8.0 were created by disodium hydrogen phosphate-citric acid buffers, pH 9.0 by borax-hydrochloric acid buffer, pH 10.0 ~ 11.0 by sodium bicarbonate-sodium hydroxide buffers, and pH 12.0 ~ 13.0 by potassium chloride-sodium hydroxide buffers<sup>[16]</sup>.

### 1.5 Determination of the checking wavelength

0.5 mL extract of the root tuber anthocyanins was added with 9.5 mL distilled water, shaken up adequately, and then scanned in the 400 ~ 700 nm range. Only one absorption peak, namely at 517.0 nm, was observed, with the maximal absorption wavelength in visible light ( $\lambda_{\text{vis max}}$ ) shifting from 530.0 to 517.0 nm which was used as the checking wavelength in the following experiments<sup>[9]</sup>.

### 1.6 Effects of pH on the visible light absorption spectra of the root tuber anthocyanins

0.5 mL extract was added with 9.5 mL solutions of pH 0.0 ~ 13.0 respectively, shaken up adequately, placed at 15 °C in darkness for 30 min, and then scanned in the 400 ~ 700 nm range. The absorbance value at 517.0 nm ( $A_{517.0}$ ), color and  $\lambda_{\text{vis max}}$  were recorded.

### 1.7 Determination of the pH-induced reversibility of the root tuber anthocyanins

0.5 mL extract was added with 9.5 mL solutions of pH 0.0 ~ 13.0 respectively, shaken up adequately, placed at 15 °C in darkness for 1 h, and then added 10% HCl drop by drop till the pH values of all solutions were adjusted to 2.0. The absorbance values at 517.0 nm of the solutions were determined as soon as possible.

### 1.8 Effects of pH on the degradation rate of the root tuber anthocyanins

0.5 mL extract was added with 9.5 mL solutions of pH 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 respectively, shaken up adequately, placed at 15 °C in darkness. According to the  $\lambda_{\text{vis max}}$ s of different pHs determined in 1.6, the absorbance values at the  $\lambda_{\text{vis max}}$ s ( $A_{\lambda_{\text{vis max}}}$ s) were mensurated weekly, and the mensuration lasted 6 weeks, namely 42 days. The retention

ratios of the root tuber anthocyanins at different pH conditions were figured out by a formula: Retention ratio =  $A_t/A_0 \times 100\%$ , and the kinetic characteristics of the degradation of the anthocyanins in the solutions of different pH values were determined by the trendline of the curve of  $\log(A_0/A_t)$  to  $t$  ( $t$  stands for the mensuration time,  $A_t$  stands for the absorbance value at Time  $t$ ,  $A_0$  stands for the primitive absorbance value).

## 2 Results and analyses

### 2.1 Effects of pH on the visible light absorption spectra of the root tuber anthocyanins of *P. notoginseng*

The effects of pH values on the visible light absorption spectra of the root tuber anthocyanins of *P. notoginseng* were systematically reflected by the color,  $\lambda_{\text{vis max}}$  and  $\Delta\lambda_{\text{vis max}}$  of the solutions.

**Tab. 1** Changes of the color and  $\lambda_{\text{vis max}}$  of the root tuber anthocyanins of *P. notoginseng* in solutions of different pH values

pH	before being adjusted to pH 2.0		after being adjusted to pH 2.0	
	color	$\lambda_{\text{vis max}}/\text{nm}$	color	$\lambda_{\text{vis max}}/\text{nm}$
0.0	red	527.1	dark red	532.2
1.0	red	530.5	dark red	532.4
2.0	crimson	532.3	crimson	532.5
3.0	light red	533.8	red	532.0
4.0	light pink	535.7	pink	532.8
5.0	pink	537.0	light pink	533.0
6.0	faint pink	542.4	wispy pink	535.7
7.0	light blue	558.2	blue	560.0
8.0	bluish green	570.0	blue	571.4
9.0	blackish green	591.2	blackish green	595.2
10.0	yellowish green, black precipitate	408.7	yellow	408.1
11.0	blackish yellow, black precipitate	-	yellow	-
12.0	blackish yellow, black precipitate	-	yellowish black	-
13.0	brown, black precipitate	-	black	-

Note: “-” shows “disappearance”.

(1) The color of the root tuber anthocyanins of *P. notoginseng* were observed to change along with the pH values. When  $\text{pH} \leq 3.0$ , the solution expressed flamboyant red, and at pH 2.0, the solution expressed the strongest red. When pH ranged from 3.0 to 6.0, the red of the solution faded rapidly. At pH 6.0, the solution was almost colorless. When pH ranged from 7.0 to 9.0, blue emerged and resulted in the bluish green at pH 9.0. When pH was higher than 9.0, the solution changed from blackish green to blackish yellow, and at pH 13.0, the solution became brown. Furthermore, when pH continued to increase from 10.0, black precipitate was observed (Tab. 1). Therefore, the coloration of the root tuber anthocyanins of *P. notoginseng* is pH-dependent. At pH 2.0, the anthocyanins expressed the strongest

red. Acidity held distinct hyperchromic effect on the color of the anthocyanins and basicity could make the anthocyanins become blue, black and brown, and produce black precipitate, which is in agreement with the findings reported by BROUILLARD<sup>[17]</sup>, PANG et al.<sup>[18]</sup> and so forth.

(2) The  $\lambda_{\text{vis max}}$  of the root tuber anthocyanins of *P. notoginseng* was also found to be pH-dependent. When pH ranged from 0.0 to 9.0, the  $\lambda_{\text{vis max}}$  increased gradually, namely expressed bathochromic shift. However, from pH 9.0 to 10.0, the  $\lambda_{\text{vis max}}$  shifted from 591.2 to 408.7, namely expressed hypsochromic shift, being accompanied by the color change from blackish green to yellowish green (Tab. 1). When  $\text{pH} \geq 11.0$ , the  $\lambda_{\text{vis max}}$  disappeared (Tab. 1), which is consistent with the result reported

by YE et al.<sup>[19]</sup>. So, along with the gradual increase of pH value from 0.0 ~ 13.0, the  $\lambda_{\text{vis max}}$  of the root tuber anthocyanins of *P. notoginseng* expressed bathochromic shift and hypsochromic shift orderly, then disappeared.

(3) The  $A_{\lambda_{\text{vis max}}}$ , namely  $A_{517.0}$ , of the solutions of the root tuber anthocyanins of *P. notoginseng* changed along with pH values. When pH increased from 0.0 to 2.0,  $A_{517.0}$  increased and reached the climax at pH 2.0, which may due to the aggregations of the anthocyanin in the forms of carbinol pseudobase or quinonoidal base<sup>[17]</sup>. When the pH continued to increase from 2.0,  $A_{517.0}$  decreased rapidly along with the rise of pH value. Finally, at pH 6.0, the absorption peak at 517.0 nm disappeared completely. When the pH value ranged from 7.0 to 13.0,  $A_{517.0}$  kept at a very low level (Fig. 1). This phenomenon is consistent with the research results of SKREDE<sup>[20]</sup>, PANG et al.<sup>[18]</sup>, YE et al.<sup>[19]</sup> and so on. As a result, if the pH value ranged from 0.0 ~ 13.0, the  $A_{\lambda_{\text{vis max}}}$  of the solutions of the root tuber anthocyanins exhibited a single-peak curve, with the peak at pH 2.0.

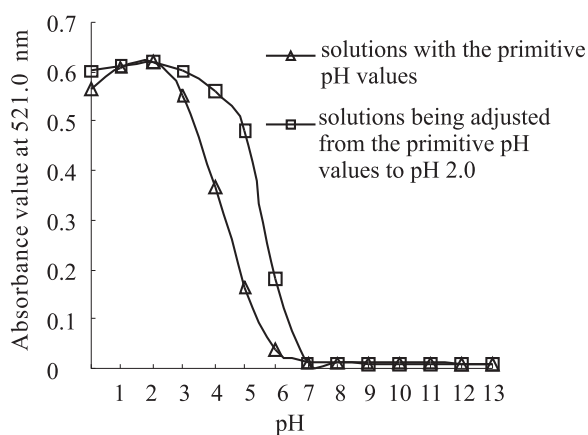


Fig. 1 Absorbance values at 517.0 nm ( $A_{517.0}$ ) of the root tuber anthocyanins of *P. notoginseng* in solutions of different pH values

Theoretically, the pH-dependent characteristics of anthocyanins are mainly because the molecular structures of anthocyanins transform along with the pH values of the solution<sup>[17,21~25]</sup>.

The oxygen atom of the pyran cycle of anthocyanins is quadrivalent, which results in the basicity and

high chemical vivacity of anthocyanins. At the same time, the phenolic hydroxyls of anthocyanins result in the acidity of anthocyanins<sup>[17,21,22]</sup>. So, in solutions, anthocyanins exist as zwitterions, and some groups of anthocyanins, such as -OH, -C=O and =O-, generate nucleophilic or electrophilic rearrangement reactions because of the action of  $H^+$  or  $OH^-$ .

At different pH values, anthocyanins may exist as 4 forms of structures: the first is the flavylium cation (red, denoted as " $AH^+$ "), the second is the anhydro-base anion, namely quinonoidal base (blue, denoted as " $A$ "), the third is the carbinol pseudobase (colorless, denoted as " $B$ "), and the fourth is the chalcone (colorless, denoted as " $C$ "). They can be transformed one another because of pH values (Fig. 2). Three equilibriums exist in the four forms of structures.

(1) Acidic equilibrium:  $AH^+$  (Red)  $\leftrightarrow$  A (Blue) +  $H^+$ . In acidic media, A turns into  $AH^+$ . Emergence of  $H^+$  accelerates the atom O in the central ring of anthocyanins to form conjugating bond with the atoms of other rings, which makes the three rings coexist in one plane. The coplanar pattern of three rings makes the structure of anthocyanins more stable (Fig. 2). On the other hand, in weak acidic and neutral media, the  $\lambda_{\text{vis max}}$  of anthocyanin solution shifts toward the long wavelength side (Tab. 1).

(2) Hydration equilibrium:  $AH^+$  (Red) +  $H_2O \leftrightarrow B$  (Colorless). In alkaline media,  $AH^+$  turns into B.  $sp^2$  orbit of  $C_2$  in the central ring of anthocyanins changes into  $sp^3$  (B), which makes the coplanar pattern of three rings, and the corresponding conjugating effect, be destroyed. The conjugated system is essentially the chromophore of anthocyanins. So, the destroy of conjugating effect is the most dominating factor which results in the loss of anthocyanin color (Fig. 2)<sup>[17,26,27]</sup>. As a whole, in alkaline media, anthocyanins exist first as anhydro-base anions which display blue hue, then the anhydro-base anions are unstable and hydrated easily to be the colorless pseudobases<sup>[24,25]</sup>.

(3) Chain-ring equilibrium: B (Colorless)  $\leftrightarrow$  C (Colorless). In the structure of B, the bond between

O and C<sub>1</sub> in the central ring can rupture, forming the intermediate between B and C. In the intermediate, there is a spatial position obstruction between the carbonyl group of C<sub>1</sub> and hydroxyl group of C<sub>9</sub>. In order to eliminate the obstruction, the benzene ring, namely Ring A, rotates along the single bond between C<sub>4</sub> and C<sub>10</sub>. The conjugating effect of the two benzene rings,

namely Ring A and B, at the ends of anthocyanins is destroyed to form the structure C, resulting in the permanent disappearance of the red hue (Fig. 2).

Moreover, the pH-dependent characteristics of anthocyanins are also because pH is directly related to the occurrence of the copigmentation of anthocyanins and copigments<sup>[17,21,22]</sup>.

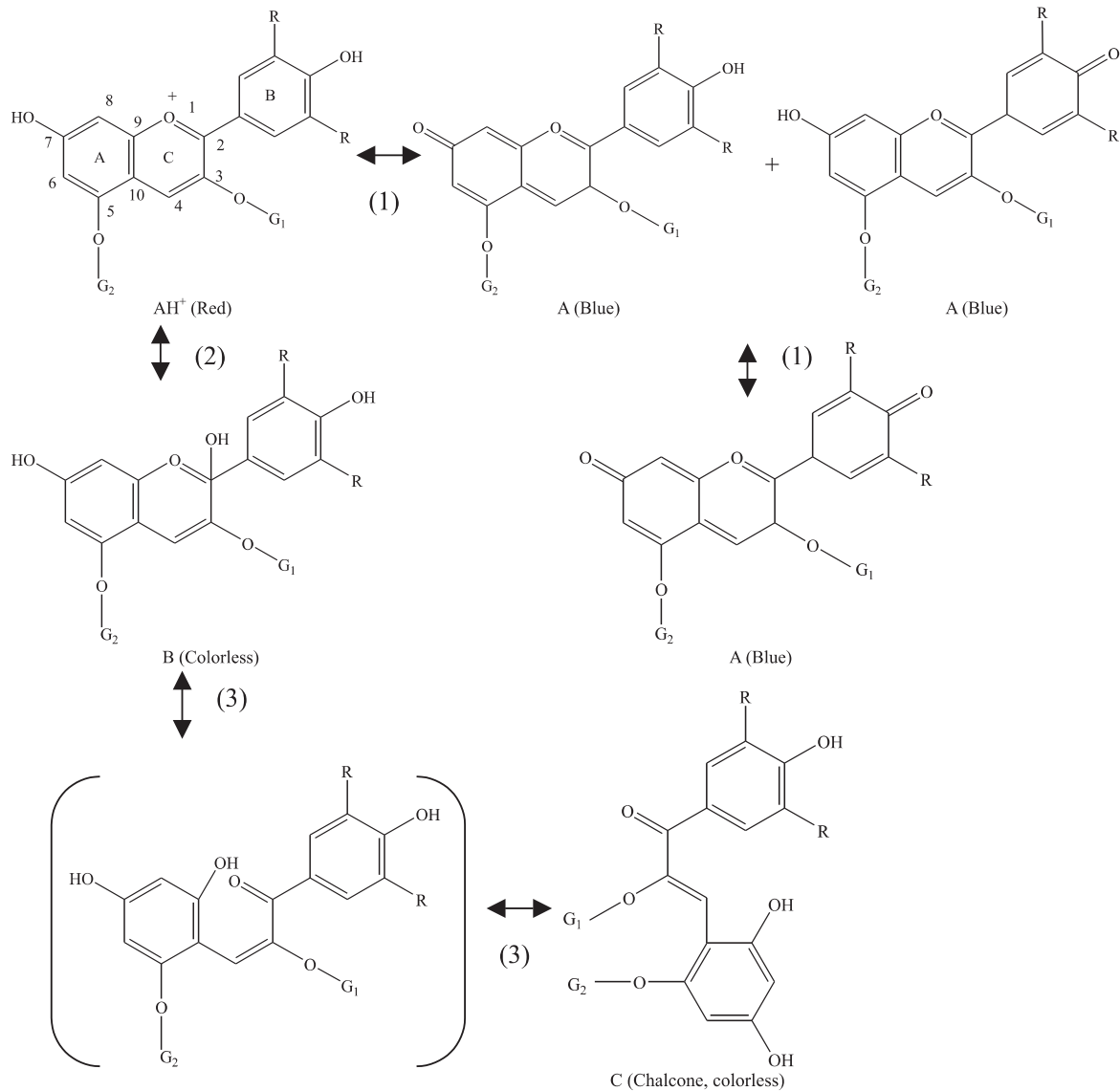


Fig. 2 Schematic illustration of the structural transformation of anthocyanins caused by pH values

(drawn according to Literature [17,21~24]. R stands for substituting group, such as OH, OCH<sub>3</sub>, etc. G<sub>1</sub> and G<sub>2</sub> stands for glycosyls).

## 2.2 pH-induced reversibility of the root tuber anthocyanins of *P. notoginseng*

After the pH values of all solutions were adjusted to 2.0 by the addition of 10% HCl, the restorable degrees of the colors,  $\lambda_{\text{vis max}}$ s and  $A_{\lambda_{\text{vis max}}}$ , namely

$A_{517.0}$ , of the solutions of the root tuber anthocyanins of *P. notoginseng* were directly related to the primitive pH values.

(1) After the pH values were adjusted to 2.0, the colors of the solutions of pH 0.0, 1.0, 3.0,

4.0, 5.0 and 6.0 all changed toward red hues, and the colors of the solutions with the primitive pH values ranging from 7.0 to 13.0 still expressed bluish purple, blue, blackish green, yellow or black, being accompanied by the disappearance of the precipitates (Tab. 1). When  $\text{pH} \leq 6.0$ , once the pH values were restored to 2.0, the red hues of the anthocyanins were all resumed to much stronger, which is consistent with the result reported by YE et al.<sup>[19]</sup>. When  $\text{pH} \geq 7.0$ , the acidification could not resume the red hues of the anthocyanins at all, even though the acidification resulted in the dissolution of the black precipitates clearly. Therefore, pH 2.0 best favored the expression of the strongest red of the root tuber anthocyanins, it was the optimum pH of the pigmentation of the root tuber anthocyanins.

(2) After the pH values were adjusted to 2.0, the  $\lambda_{\text{vis max}}$ s of the solutions of pH 0.0, 1.0, 3.0, 4.0, 5.0 and 6.0 were almost resumed to 532.0 nm. On one hand, the  $\lambda_{\text{vis max}}$ s of the solutions of pH 0.0 and 1.0 expressed bathochromic shift, namely from 527.1 nm to 532.2 nm and from 530.5 nm to 532.4 nm respectively. On the other hand, the  $\lambda_{\text{vis max}}$ s of the solutions of 3.0, 4.0, 5.0 and 6.0 expressed hypsochromic shift, namely from 533.8 nm to 532.0 nm, from 535.7 nm to 532.8 nm, from 537.0 nm to 533.0 nm and from 542.4 nm to 535.7 nm respectively (Tab. 1). This may also due to the aggregations of the anthocyanins in the forms of carbinol pseudobase or quinonoidal base<sup>[17]</sup>. However, when the primitive pH values  $> 6.0$ , the  $\lambda_{\text{vis max}}$ s of the solutions did not almost change, being gone with the permanent disappearance of the red hue (Tab. 1). Thus, if the primitive pH values  $< 6.0$ , after the pH values were resumed to 2.0, the  $\lambda_{\text{vis max}}$ s of the solutions of the root tuber anthocyanins altered to 532 nm in different degrees.

The fact that the lost red hues of anthocyanins can be partly restored by the “optimum pH” is due to the incomplete structural transformation of anthocyanins from C to  $\text{AH}^+$  (Fig. 2). In the course of pH restoration from the primitive values to 2.0, the central ring of anthocyanins may gradually form again,

leading the structural shift from the intermediate between B and C to  $\text{AH}^+$ , subsequently from C to  $\text{AH}^+$ . But because of the spatial position obstacle, not all of C can be shifted to  $\text{AH}^+$ , which accounts for the reason why the red hue can not be recovered entirely<sup>[21,22]</sup>.

(3) After the pH values were adjusted to 2.0, if the primitive pH values of the solutions  $\leq 6.0$ , the  $A_{\lambda_{\text{vis max}}}$ , namely  $A_{517.0}$ , of the solutions were found to increase, and if the primitive pH values of the solutions  $\geq 7.0$ , the  $A_{517.0}$ s remained unchangeable, resting on the very low values (Fig. 1).

Based on the above analyses, it could be reasonably concluded that the pH values which were lower than 6.0 did not destroy the molecular structures of the root tuber anthocyanins of *P. notoginseng*, the pH values which were higher than 6.0 demolished the molecular structures of the anthocyanins, being evidenced by the perpetual loss of the red hues, the non-restorability of the  $\lambda_{\text{vis max}}$ s and the non-increase of the  $A_{\lambda_{\text{vis max}}}$ s of the solutions of the anthocyanins.

### 2.3 Effects of pH on the degradation rate of the root tuber anthocyanins of *P. notoginseng*

Despite being placed at 15 °C in darkness, after 42 days, the root tuber anthocyanins of *P. notoginseng* in solutions of different pH values all degraded in different speeds, being indicated by the fact that the colors faded in different degrees (Tab. 2).

**Tab. 2 Color changes of the root tuber anthocyanins of *P. notoginseng* in solutions of different pH values along with time**

pH	color	
	0 day	42 days later
0.0	red	light red
1.0	red	light red
2.0	crimson	red
3.0	light red	faint red
4.0	light pink	faint pink
5.0	pink	wispy pink
6.0	faint pink	colorless

The degradation rates of the root tuber anthocyanins in solutions of different pH values were obviously

different. The degradation rate in the solution of pH 2.0 was the slowest, and the rate in the solution of pH 6.0 was the quickest. When the  $pH \leq 3.0$ , the anthocyanins degraded slowly as a whole, and when the  $pH \geq 4.0$ , the anthocyanins degraded rapidly (Fig. 3). Hereby, the low pH values which ranged from 0.0 to 3.0 were propitious to the stable storage of the root tuber anthocyanins, and pH 2.0 was the most appropriate one for the long time storage of the anthocyanins.

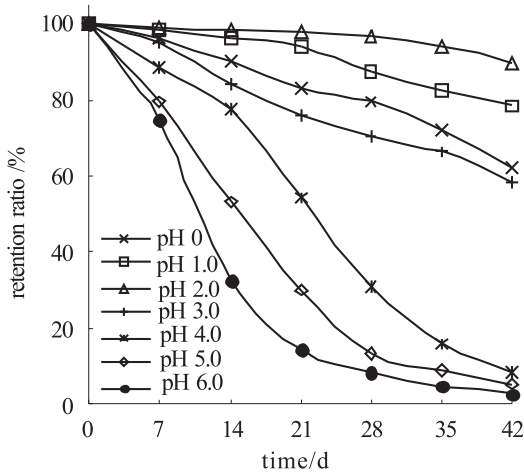


Fig. 3 Degradation rates of the root tuber anthocyanins of *P. notoginseng* in solutions of different pH values

The kinetic characteristic of the degradation of the root tuber anthocyanins in different pH conditions could be elementarily reflected by the trendline of the curve of  $\log(A_0/A_t)$  to  $t$ . In the course of degradation of the anthocyanins along with time, the curves of  $\log(A_0/A_t)$  to  $t$  at different pH values were almost straight lines (Fig. 4), suggesting that the degradation courses of the root tuber anthocyanins in different pH conditions approximated first-order reaction kinetics and the degradation might result from the molecular fissions of the anthocyanins<sup>[28]</sup>.

### 3 Discussion

All the while, ginsenosides were believed to be the most crucial medicinal ingredient of *P. notoginseng*<sup>[10,11]</sup>. However, both ginsenosides and anthocyanins have been revealed to possess various pharmacological activities. For the root tuber *P. notoginseng*, the genuine relationship of ginsenosides and

anthocyanins is obviously an interesting question, although it is still a puzzle today. In 2001, the total ginsenosides content of the purple root tuber was found to be 48.52% higher than that of the green root tuber, suggesting that the medicinal quality of the purple root tuber is better than that of the green root tuber<sup>[7]</sup>. In 2007, we found that the anthocyanin content of the root tuber is positively related with the total ginsenosides content at the significant level<sup>[9]</sup>. These two evidences seem to show that, in the root tuber *P. notoginseng*, there is a mutually beneficial interaction between the anthocyanins and the ginsenosides, and the anthocyanins appear to accelerate the accumulation of the ginsenosides. Nevertheless, the biosynthetic pathway of anthocyanins has nothing to do with that of ginsenosides<sup>[29-32]</sup>. So, the internal mechanism of the mathematical correlation between the content of the ginsenosides and that of the anthocyanins in the tuber of *P. notoginseng* is unclear at all, further studies are urgently necessary.

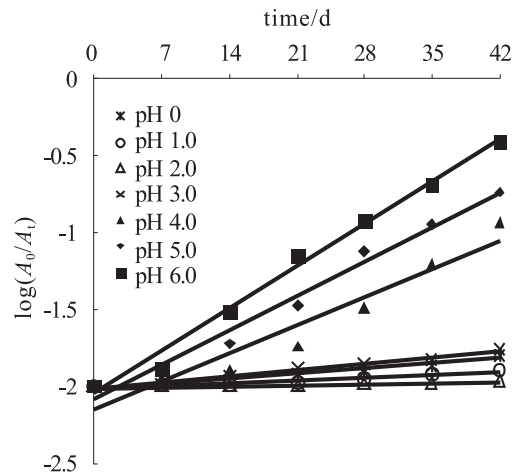


Fig. 4 Kinetic characteristic of the degradation of the root tuber anthocyanins of *P. notoginseng* in solutions of different pH values

The discovery that the purple of the root tuber results from anthocyanins may provide a totally new growing point for the industrialization development of *P. notoginseng*. The insufficient basic researches have led to the severe dispersion, chaos and promiscuity in the product-exploiting of *P. notoginseng*<sup>[33]</sup>. Exploitation of new product plays a key role in promoting *P. notoginseng* to participate in the international com-



petition<sup>[11,34]</sup>. As a natural pigment and a good alternative of synthetic colorants, anthocyanins have been widely applied in food, cosmetic and medicine industries<sup>[12]</sup>. In this study, we dealt with the detailed influences of pH on the coloration and degradation of the root tuber anthocyanins of *P. notoginseng*, suggesting that the tuber anthocyanins may be firstly and directly used as the colorant in acidic foods, such as syrup, jelly and ice cream. Now, we have established the specific extracting technology for the tuber anthocyanins and we are exploring the particular pharmacological activities of anthocyanins (data not shown), which will create a span-new territory for the modernization of *P. notoginseng*.

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