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理化因子导致梅花'南京红'花色色素的颜色变化

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摘 要：梅花是中国的候选国花之一。属于花色苷的梅花'南京红'花色色素用含1%浓盐酸(v/v)的甲醇提取，并呈现纯净的紫红色。体外试验表明：该色素在pH0～3范围内颜色稳定，因不同光质、热、氧化剂、还原剂、螯合剂而呈现无色、墨绿色或黄绿色，因不同金属离子、离子的不同浓度而呈现程度不同的红色、紫色、黑黄色、红中带黑或微蓝绿色，葡萄糖和低浓度苯甲酸钠几乎不影响其色泽，蔗糖使颜色变淡，柠檬酸却使其颜色变深。该文可为梅花红色花色的机理探索、梅花花色苷的分子结构鉴定、梅花红色花色色素的开发利用提供参考和前提。

关键词：颜色变化；花色色素；'南京红'；理化因子

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Changes in coloration of the flower color pigment of *Prunus mume* 'Nanjing Hong' (Nanjing red) caused by physicochemical factors

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Abstract: Mei(*Prunus mume* Sieb. et Zucc.)flower is one of the candidates of the national flower of China. Belonging to anthocyanin, the flower color pigment of *P. mume* Sieb. et Zucc. 'Nanjing Hong'(Nanjing red) can be extracted with methanol containing 1% concentrated HCl(v/v)and the pigment in the extraction solution expresses purely mauve. Experiments *in vitro* reveal that the pigment is stable at pH0～3. The pigment appears colorless, blackish green or yellowish green because it is sensitive to light, heat, oxidant, reductant and chelating agent. It also expresses various red, purple, blackish yellow, blackish red or faint bluish green because of different metal ions or different concentrations of the ions. Glucose and low concentrated sodium beneoate almost have no effects on the coloration. Sucrose can weaken the color, but citric acid can strengthen it. This paper could be a reference or a premise for the exploration on the flower color mechanism, the identification of the molecular structures of the anthocyanins and the exploitation and utilization of the flower color

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pigments of red Mei flowers.

Key words: Changes in coloration; Flower color pigment; *P. mume* Sieb. et Zucc. 'Nanjing Hong'(Nanjing red); Physicochemical factors

Mei (*Prunus mume* Sieb. et Zucc.) flower is one of the candidates of the national flower of China, it symbolizes the characteristics of hardiness and staunchness of Chinese in facing adversity and evil. Mei flower is native to China and is highly admired in China for its beautiful colors, various tree patterns, wispy fragrance, graceful gestures and glamourous charm. In fact, the resistance of Mei to cold and snow makes it one of the wonders of the botanical world. So far in the world, Mei flower is the unique horticultural plant which is accredited Chinese scientist, namely Chen Jun-Yu (Senior academician of Chinese Academy of Engineering, Professor of Beijing Forestry University), as International Cultivar Registration Authority (Zhao and Guo, 2003).

The sixty years of scientific research on Mei flower in China has obtained plentiful and substantial achievements (Chen, 2002), but the study on the flower color is almost a blank. Flower color refers to the color of all structures like petals in the flower organs of phanerogam which mainly points to angiosperm owing conspicuous flowers (Qi, 1989; Cheng, 2000). It has well been confirmed that the cultivars of Mei flower cultivated in China are classified into 3 series, 5 groups and 18 forms, and the flower color of Mei includes mauve, pink, white, greenish white, light yellow and double color (Chen, 2001). *P. mume* Sieb. et Zucc. 'Nanjing Hong'(Nanjing red) which belongs to Form Pink Double is the typical representative of the pink and we have confirmed its flower color pigment belongs to flavonoids and the red results from its anthocyanins. It's well known that the color of anthocyanin will change according to concrete pH, Vc, light, metal ion, temperature, H_2O_2, saccharides and their degradation products, etc (Sondheimer and Kertesz, 1953; Daravings and Cain, 1965; Wrolstad and Erlandson, 1975; Sweeny *et al.*, 1981; Skrede, 1985). However, the color changes of the anthocyanins of different sources are not unanimous (Pang *et al.* 2001; Ren *et al.* 2002).

It is first reported in this paper that the changes in coloration of the flower color pigment of *P. mume* Sieb. et Zucc. 'Nanjing Hong'(Nanjing red) caused by physicochemical factors, which could serve for the exploration on the flower color mechanism and the identification of the molecular structures of the anthocyanins and the exploitation and utilization of the flower color pigments of red Mei flowers.

1 Materials and methods

1.1 Plant material

All flowers were obtained in the Research Centre of Mei flower of Sun Yat-sen Mausoleum Administrative Office of Nanjing. During the full florescence, the blooming flowers were collected randomly in the morning on March 9 of 2003 just after the dew evaporated completely, encased in ordinary kraft envelopes, and immediately frozen at -20 ~-22 ℃ until extraction.

1.2 Isolation of the flower color pigment

Away from androecia, the frozen petals were rapidly pulled out with stainless steel tweezers. 8 g petal was ground in a white porcelain pestle quickly and completely at room temperature after mixing with approximate 15 mL methanol containing 1% concentrated HCL (v/v) (Markham, 1982). Extracts were filtered and the residue was washed till it became full white. The filtrate was primarily purified by partition against n-hexane, and the final extract was diluted to 100 mL with the above acidic methanol, and it expressed purely mauve. The extract was refrigerated under 3 ℃ in darkness until analysis.

1.3 Changes in coloration of the flower color pigment caused by physical factors

The extract was diluted properly and the absorption spectrum was recorded at room tempera-

ture in a 1 cm pathlength quartz cell in the 200～700 nm range using a SHIMADZU UV-VIS spectrophotometer. Three absorption peaks, namely 530.0, 339.0 and 250.0 nm, were observed. 530.0 nm was regarded as the checking wavelength in the following experiments because it had been proved to be the specific absorption of anthocyanins (Rabino and Mancinelli, 1986; Takeda *et al.* 1996; Leng and Qi, 2003).

The effects of light and temperature on the coloration were checked respectively. As to light, four treatments were designed (the temperature was 18～20 ℃): sunlight (under cloudless fine day), ultraviolet light (40 W, 50 cm from the light tube), light of fluorescent lamp (40 W, 2.5 m from the light tube), light of incandescent lamp (40 W, 2.5 m from the light bulb). A_{530} was determined hourly and the determination lasted for seven hours continuously. As to temperature, under the indoor light of fluorescent lamp, 25, 35, 45, 55, 65, 75, 85 and 95 ℃ were designed in a constant temperature water bath. The solution was sampled hourly and cooled to room temperature rapidly under tap water, the correspondent A_{530} determination lasted for seven hours continuously.

1.4 Changes in coloration of the flower color pigment caused by chemical factors

As to pH, 1 mL extract was placed in every securely stoppered test tube, added 9 mL solutions of pH0.0～8.0 respectively, balanced in darkness after being shaken up, and then scanned in the 200～700 nm range. The maximal absorption wavelength in visible light ($\lambda_{vis\ max}$) and the correspondent absorbance were recorded. Thereinto, pH 0.0 was created by 1.00 mol/L HCl, pH1.0 by 0.10 mol/L HCl, pH2.0 by 0.01 mol/L HCl, pH 3.0～8.0 were created by a series of disodium hydrogen phosphate-citric acid buffers.

As to metal ions, oxidant, reductant, chelating agent, saccharide, preservative and citric acid, 1 mL extract was added with 9 mL H_2O, and scanned in the 200～700 nm range. Two absorption peaks, namely 514.0 and 324.0, were observed, with the $\lambda_{vis\ max}$ shifting from 530.0 nm to 514.0 nm which was regarded as the checking wavelength in the following experiments. For every reaction of different chemical factors, 1 mL extract was added with 9 mL solution of special concentration, the corresponding color changes and A_{514} were noted. Metal ions dealt with Al^{3+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Ca^{2+}, Sn^{2+}, Co^{2+}, Pb^{2+}, K^+ and Na^+. Hydrogen peroxide was used as oxidant, Vc and sodium sulfite as reductants, ethylenediaminetetra-acetic acid (EDTA) (melt in 1% sodium carbonate) as chelating agent, and the roles of glucose, sucrose, citric acid and sodium beneoate were testified respectively.

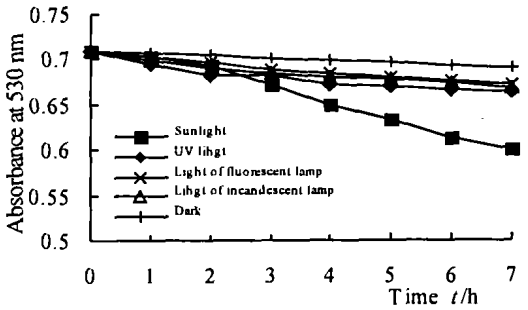

Fig. 1 The effects of light on the stability of the flower color pigment of *P. mume* 'Nanjing Hong' (Nanjing red)

2 Results and analyses

2.1 Changes in coloration of the flower color pigment caused by light and temperature

The pigment coloration is light-unstable. Sunlight, ultraviolet light, lights of fluorescent lamp and light of incandescent lamp all resulted in its degradation, showing the red became light and A_{530} decreased continuously (Fig. 1). Furthermore, the effect of sunlight was the strongest, ultraviolet light was less stronger, and the effects of light of fluorescent lamp and incandescent lamp were both smaller and consistent (Fig. 1). Thus, the pigment is sensitive to light, which is the most obvious characteristics of anthocyanins (Sweeny *et al.* 1981).

The resistance of the pigment to heat is very weak. Up to 35 ℃, A_{530} decreased steeply along

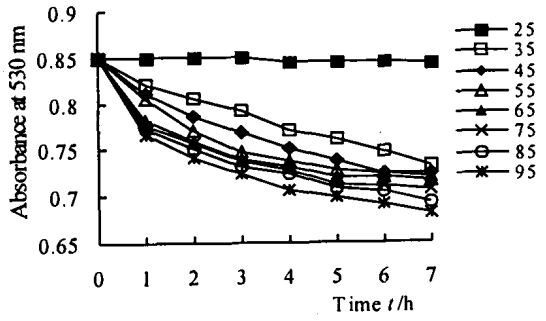

Fig. 2 The effects of temperature on the stability of the flower color pigment of *P. mume* 'Nanjing Hong' (Nanjing red)

with time. The higher the temperature was and the longer the treatment time was, the thinner the pigment's red was (Fig. 2). This is probably because temperature influences the co-pigmentation of anthocyanins and co-pigments (Mazza and Brouillard, 1987), and the high temperature also results in the conversion from anthocyanin to colorless chalcone (Brouillard, 1982), although the conversion is reversible (Mazza and Brouillard, 1987).

2.2 Changes in coloration of the flower color pigment caused by chemical factors

The coloration of the pigment is pH-dependent. It was red at pH0～3.0, the red became thin till colorless at pH5.0～6.0, and black emerged gradually when pH > 6.0. The absorption peak disappeared when pH=5.0, but the correspondent A $\lambda_{vis\ max}$ reached the climax at pH1.0 and subsequently decreased along with the ascending of pH (Table 1), which is consistent with the research results of Skrede G. (1985) and Pang X. Q. *et al.* (2001). The pigment color was stable at low pH, and changed when pH varied from weakly acidic to neutral, expressing the most important characteristics of anthocyanins that their colors change along with pH (Brouillard, 1983). This is because pH is directly related to the occurrence of the copigmentation of anthocyanins and copigments. At the same time, the pH effects analyzed above implied that the pigment is non-acylated or mono-acylated (Brouillard, 1983; Mazza and Brouillard, 1987).

Table 1 The changes in coloration of the flower color pigment of *P. mume* 'Nanjing Hong' (Nanjing red) caused by pH (The average value of two experiments)

pH	0	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Color	red	red	red	light red	light pink	wispy red	colorless	wispy black	light black
$\lambda_{vis\ max}$	516.0	515.0	515.0	515.0	514.0	—	—	—	—
A $\lambda_{vis\ max}$	0.6961	0.7090	0.6628	0.4954	0.3102	—	—	—	—

Annotation: "—" shows "disappearance"

The coloration of the pigment varies along with metal ions and their concentrations. Al^{3+}, Co^{2+}, K^+, Na^+ all could make the pigment maintain red and the higher the concentrations of metal ions were, the stronger the red became. Cu^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}, Ca^{2+} could all also make the pigment maintain red, but the concentrations of metal ions were contrarily related to the degree of the red. Fe^{3+} made the pigment change from red to black. Fe^{2+} made it blackish red. Sn^{2+} made it flamboyant purple, but the higher the concentration was, the weaker the purple was. Lower concentrated Pb^{2+} made the pigment maintain red, the slightly high concentration (0.01 mol/L) made the pigment light blue, then turned colorless, and the much higher concentration made the pigment become wispy blue green (Table 2). The reason that metal ions influence the coloration of anthocyanins is probably that Sn^{2+} (Salt and Thomas, 1957), Cu^{2+} (Somaatmadja *et al.*, 1964), Al^{3+} (Jurd and Asen, 1966) can form stable metallo-anthocyanins complexes, Mg^{2+}, Fe^{3+}, Fe^{2+}, K^+ are related to the structural stability of acylated anthocyanins (Takeda and Hayashi, 1977; Takeda, 1977), although the stability of metallo-anthocyanins per se may not require metal ions (Hoshino *et al.*, 1980), and Ca^{2+}, Fe^{3+}, Fe^{2+}, Al^{3+} hold some protection effects to ordinary anthocyanins, though the complexes formed

also decomposes along with time (Mazza and Brouillard, 1987).

The pigment is sensitive to oxidant and reductant. 1% of hydrogen peroxide made the pigment change from red to colorless, which was reflected by the steep decrease of A_{514}. Ascorbic acid made the pigment color thin, and the higher the concentration was, the lower the corresponding A_{514} was. Sodium sulfite (also regarded as preservative in food processing) could destroy the pigment mightily, showing that the low concentration (approximate 0.125%) resulted in the abrupt decrease of A_{514}. The 0.25% of sodium sulfite made the pigment change from wispy red to colorless. The 0.5%～8.0% resulted in yellowish green, and the higher the concentration was, the stronger the yellowish green was, but the corresponding A_{514} didn't change greatly any more (Fig. 3).

Table 2 The changes in coloration of the flower color pigment of *P. mume* 'Nanjing Hong' (Nanjing red) caused by metal ions (The average value of two experiments)

ion	Concentration ($\times 10^{-3}$ mol/L)	A_{514}	color	ion	Concentration ($\times 10^{-3}$ mol/L)	A_{514}	color
Al^{3+}	0	0.768	red		50.00	0.761	red
	3.125	0.673	light red		200.0	0.746	light red
	12.50	0.694	red	Ca^{2+}	0	0.768	red
	50.00	0.708	red		3.125	0.753	red
	200.0	0.777	strong red		12.50	0.745	red
Fe^{3+}	0	0.768	red		50.00	0.741	red
	3.125	0.544	light blackish yellow		200.0	0.734	light red
	12.50	0.546	light blackish yellow	Sn^{2+}	0	0.768	red
	50.00	0.559	blackish yellow		0.3125	0.638	purple
	200.0	0.648	golden yellow		1.250	0.591	purple
Cu^{2+}	0	0.768	red		5.000	0.577	purple
	0.4000	0.799	red		20.00	0.571	purple
	1.600	0.774	red	Co^{2+}	0	0.768	red
	6.400	0.771	red		3.125	0.704	red
	25.60	0.766	light red		12.50	0.837	red
Zn^{2+}	0	0.768	red		50.00	0.981	strong red
	3.125	0.776	strong red		200.0	1.671	blackish red
	12.50	0.770	red	Pb^{2+}	0	0.768	red
	50.00	0.760	red		0.6250	0.776	red
	200.0	0.724	light red		2.500	0.757	red
Mg^{2+}	0	0.768	red		10.00	0.611	from light blue to colorless
	3.125	0.780	strong red		40.00	0.403	wispy bluish green
	12.50	0.772	red	Na^{+}	0	0.768	red
	50.00	0.769	red		3.125	0.774	red
	200.0	0.736	light red		12.50	0.782	red
Fe^{2+}	0	0.768	red		50.00	0.786	red
	3.125	0.798	red		200.0	0.792	strong red
	12.50	0.806	wispy blackish red	K^{+}	0	0.768	red
	50.00	0.841	light blackish red		6.250	0.769	red
	200.0	0.934	blackish red		25.00	0.781	red
Mn^{2+}	0	0.768	red		100.0	0.784	red
	3.125	0.794	strong red		400.0	0.789	strong red
	12.50	0.778	red				

Low concentration of EDTA made the pigment be blackish green, showing that the coloration of the pigment is certainly involved with metal ions. But it was unknown why the 1% contrarily made the pigment be light red (Table 3).

The coloration of the pigment also has something to do with citric acid and sodium beneoate. Citric acid might maintain the red and the higher its concentration was, the stronger the color was (Table 3), which implied the color of Mei flower is probably related to Tricarboxylic Acid Cycle. Sodium beneoate was often used as preservative in food

processing, and the low concentration (<0.025%) could hardly affect the coloration of the pigment, the comparatively higher concentration (>0.1%) led to light red till colorless (Table 3).

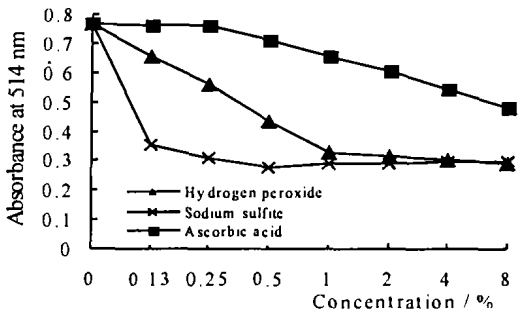

Fig. 3 The effects of oxidant and reductant on the stability of the flower color pigment of *P. mume* 'Nanjing Hong' (Nanjing red)

Table 3 The changes in coloration of the flower color pigment of *P. mume* 'Nanjing Hong' (Nanjing red) caused by chelating agent, sodium beneoate and citric acid (The average value of two expriments)

reagent	concentration(%)	A_{514}	color
$[CH_2N(CH_2COOH)_2]_2$	0	0.768	red
	0.015 625	0.572	blackish green
	0.062 500	0.567	light black
	0.250 00	0.456	wispy black
	1.000 0	0.561	light red
C_6H_5COONa	0	0.768	red
	0.025	0.764	red
	0.10	0.757	light red
	0.40	0.467	wispy red
	1.6	0.366	colorless
$C_8H_8O_7 \cdot H_2O$	0	0.768	red
	0.10	0.770	red
	0.40	0.772	red
	1.6	0.775	strong red
	6.4	0.791	strong red

It was known that glucose and sucrose both *in vivo* influence the synthesis and accumulation of anthocyanin (Qi, 1989; Weiss *et al.*, 1992). However, it was found in the experiment that glucose almost didn't influence the coloration of the pigment and sucrose contrarily resulted in the light color. The pigment was still red in the solution of glucose and sucrose. When the concentration was lower than 0.312 5%, both of them almost resulted in the decrease of A_{514} at the same degree. But it was not obvious that the color difference was induced by different concentrations of glucose, reflecting the comparatively small change of A_{514}. On the contrary, the increase of sucrose concentration (>0.312 5%) resulted in the lighter and lighter red and continuous descending A_{514} (Fig. 4).

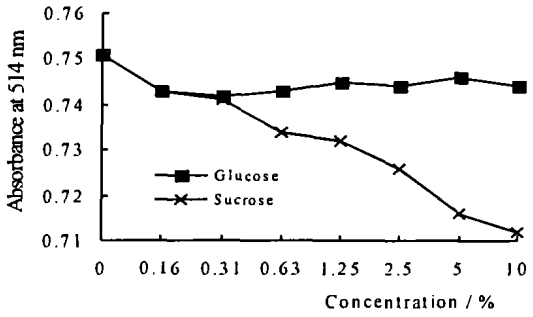

Fig. 4 The effects of glucose and sucrose on the stability of the flower color pigment of *P. mume* 'Nanjing Hong' (Nanjing red)

3 Dicussion

The flower color pigment of *Prunus mume* Sieb. et Zucc. 'Nanjing Hong' (Nanjing red) is purely mauve in methanol containing 1% concentrated HCl (v/v). However, light, heat, metal ion, oxidant, reductant, chelating agent, saccharide, citric acid and preservative all affect the coloration of the pigment, which can be the premise of the pigment-purifying and structure-elucidating and also benefits the percipience of the coloration behavior of the pigment *in vivo* (Brouillard R, 1983).

We have understood that the flower color pigment of red Mei flower is the red anthocyanins and the non-red flavonoids, the later are probably thought to be copigments which not only contribute to the red (Mazza and Brouillard, 1987) but also protect anthocyanins from various lights (Sweeny *et al.*, 1981). Furthermore, the flavanoids also makes anthocyanins avoid the attack of nucleophiles and be stable in the cell circumstance (Brouillard, 1983).

It has been a long history that Mei flower was eaten by Chinese (Ning, 1999). Modern people,

particularly the urban citizens, have been longing for natural foods due to paying attention to their health and life quality, which indicates the exploration and utilization of edible Mei flower and its red pigment will create tremendous economic and social values. The flower color pigment of *Prunus mume* Sieb. et Zucc. 'Nanjing Hong' (Nanjing red) can be used as the natural dyeing reagent of acidic food and cold beverage because of its natural and sightly luster, together with its pharmacological functions (Xiao and Lu, 1987). This research can provides theoretical bases for the usage of the food additives such as preservative, citric acid in this pigment and the selection of processing conditions and package materials.

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