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PHYSICO-CHEMICAL PROPERTIES OF PIGMENT IN GARDEN PANSY (*VIOLA* × *WITTROCKIANA* GAMS.)

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Abstract

Pansy is a good potential resource for natural pigments due to its rich flower colours. In the present study, pigment from a pansy breeding line, E01, was extracted and its solubility in different reagents and stability under various environmental conditions were investigated. The pigment extracted displayed water-soluble property and was also dissolved in acid (HCl) and alkaline (NaOH) reagents and relatively polar solvents, but insoluble in nonpolar solvents. The pigment-water solution had an absorbance peak at 238 nm with amaranthine colour under acidic conditions. It was stable in aqueous solution under normal conditions. However, it was easily spoiled when exposed to high pH (> 4), high temperature (> 80°C) or under intense light exceeding 170000 lx. The stability of pigment was significantly influenced by Cu^{2+}, but scarcely affected by Na^{+}, Mg^{2+} and Ca^{2+}. These results will be helpful for commercial prospects of pansy pigment in natural food colour industry.

Introduction

Food colouring is important in the food industry with the development of modern science and technology, many kinds of pigments were synthesized and widely used for food colouring. However, the synthetic pigments could be harmful to human health. Compared to synthetic pigments, natural anthocyanins have less side effects and showed greater commercial value in food and cosmetics (Francis 1989, Bridle and Timberlake 1997). Thus, natural pigments have become more and more popular and gradually replacing synthetic pigments (Wissgott and Bortlik 1996). Finding new source of natural pigments for food colourant has attracted widespread attention ~~at~~ worldwide (Francis 1987, Rodrýguez-Saona *et al*. 2006).

Garden pansy (*Viola* ×*Wittrockiana*) is a bedding flower widely used in landscaping (Li *et al*. 2014). From the early years of the 19th century, more and more pansy cultivars have been bred with abundant flower colours (Yoshioka *et al*. 2006). The flower of garden pansy has shown an increasing trend as food decorations because of its rich anthocyanins content and antioxidant capacity (Vukics *et al*. 2008) in addition to its various flower color appearance. Moreover, pansy has been long used as a traditional herb to treat various skin disorders, upper-respiratory problems, and a diuretic in some countries (von Bruchhausen *et al*. 1993). Therefore, garden pansy would be a good resource of natural pigments and a great anthocyanin pool.

But the knowledge about physico-chemical properties of pigments in garden pansy is very limited till now. Thus in the present study, pigment from a pansy breeding line E01 was extracted and its physico-chemical stability was investigated. The results provide preliminary knowledge for the pansy pigments extraction and application.

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Materials and Methods

The pansy breeding line, E01, with abundant purple flowers and early flower phenotype (Fig. 1a), was grown in the horticultural farm of Henan Institute of Science and technology (Latitude: 35.18° N, Longitute: 113.55° E), Xinxiang, Henan Province, China. The extraction of pigments was performed as described by Zou *et al*. (2014) with some modifications. A total of 1.0 g air-dried flower petals powder was homogenized with 5 ml 1% hydrochloric acid-ethanol. The mixture was quickly transferred into a 10 ml test tube and vortexed for 10 s. After extraction for 1 hr at room temperature (18°C), the mixture was centrifuged at 10000 × g for 15 min and the purple supernatant was transferred and condensed by decompressing distillation process below 80°C. After that, the concentrates were evaporated to black paste in a rotary evaporator at 60°C. Finally, the black pastes were dried in a drying box (70-80°C) and weighed.

Eleven solvents including water, 95% ethanol, absolute methanol, n-butyl alcohol, acetone, ethyl acetate, chloroform, tetrachloromethane, petroleum ether, 0.1 M HCl, 0.1 M NaOH, were used to evaluate the solubility of pansy flower pigments. To evaluate the dissolution of pigments, solid pigments sample of 0.1 g was put in 3 ml of each solvent, respectively, and kept at 18°C for 3 hrs. All the treatments were repeated three times.

Solid pigments sample of 0.2 g was dissolved in 500 ml distilled water. The spectral characteristic of the pigments was determined using a spectrophotometer (TU1901, China) (Giusti and WroIstad 2001), and distilled water was used as the control.

Effects of pH, temperature, light and metallic ions on the stability of pigments were evaluated using the pigment-water solution of 0.2 g/500 ml. The stability of pigment-water solution was determined by colour change visually and measurement of the absorbance of the solution (Bordignon-Luiz *et a*l. 2007) in different treatments at 236 nm using a spectrophotometer (TU1901, China). All the treatments were repeated three times.

Various pH conditions ranging from 1.0 to 12.0 were set by a pH meter in 20 ml pigment-water solution adjusted by 0.1 M HCl and 0.1 M NaOH according to Xu *et al.* (2002). The colour change of pigment-water solution under different pH condition was observed and the maximum absorption was measured. Heat degradation studies were performed as described previously (EI Gharras *et al*. 2008). The 10 ml pigment-water solution was bathed in water for 1 hr at different temperature of 25, 40, 60, 80 and 100°C. After cooling room temperature to 18°C the absorption of the solution was measured using a spectrophotometer at 236 nm (TU1901, China). The pigment-water solution at room temperature was used as the control. According to Xu *et al.* (2002), light treatments were conducted under indoor sunlight (140 lx), outdoor sunlight (17000 lx) and Ultraviolet light (1000 lx). After 6 hrs illumination, the absorbance (A) of the treatment samples was measured. The pigment-water solution in kept in dark was used as the control. Different metal salts such as NaCl, $CaCl_2$, $MgC1_2$, $MnC1_2$ and $CuCl_2$ were used to evaluate the effect of metallic ions on stability of pansy pigments. A total of 10 ml mixed solutions, including 2 ml of 0.5 M metallic salts mentioned above and 8 ml pigment-water solution, were rested for 30 min at room temperature and then the absorbance (A) was measured at 236 nm. The pigment-water solution without treatments was used as the control.

The comparison of absorbance among treatments was completed using one-way analysis of variance (ANOVA) and Tukey's post hoc tests using DPS7.55 software.

Results and Discussion

Different plant-derived pigments have different solubility in different solvents (Song *et al.* 2022). To understand the solubility characteristics of pansy pigments in different solvents, 11 common extraction solvents including organic, inorganic, acid, and alkaline reagent were used to

evaluate the dissolution of pansy pigments from E01. The pansy pigment was soluble in acid (HCl), alkaline (NaOH) reagents and relatively polar solvents, including water, methanol, alcohol, *n*-butyl alcohol, acetone and Ethyl acetate (Table 1). However, it was insoluble in chloroform, tetrachloromethane and petroleum ether which are nonpolar solvents.

UV-VIS spectrum of pigments extracts from E01 by water exhibited only one absorption peak in -UV region (Fig. 1). The absorption peak was at about 236 nm. In the visible wavelength range, no apparent absorption peak was observed. So the maximum absorption value at 236 nm was chosen for pigment stability in further studies.

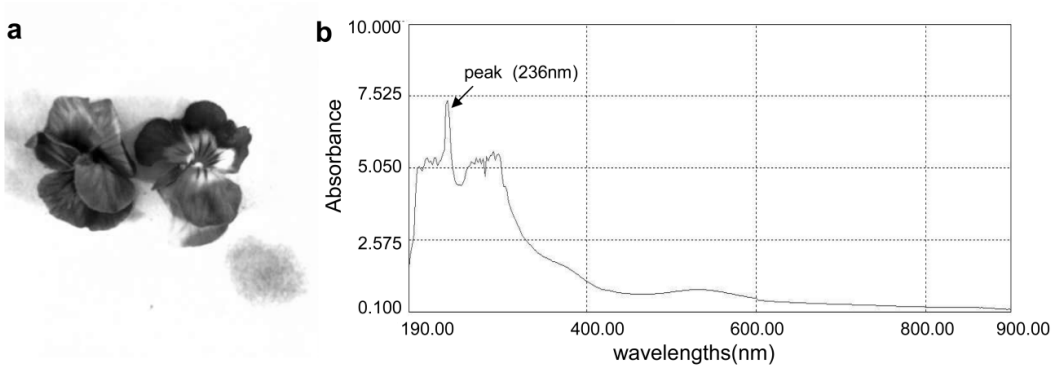

Fig. 1. The absorbance UV-VIS spectrum of pigment extracts from E01line. a. E-01-X-1 line; b. The absorbance UV-VIS spectrum of pigment extracts from E01 line.

The stability assay of pansy pigments could provide theoretical foundation for its extraction technology. Bordignon-Luiz *et al.* (2007) showed that pH value had a significant effect on the stability of pigments. The present study indicated the colour of pansy pigment aqueous solutions remained violet red and the absorbance values (A) at 236 nm were almost consistent with that of the control, when pH values ranged from 1.0 to 3.0. As the pH increased from 4.0 to 12.0, the color of the solutions varied greatly and the absorbance at 236 nm increased. When the pH value was over 8.0, the colour became yellow (Table 2). So pansy pigment was sensitive to pH values, especially when the pH > 4.0 (Table 2). These results are consistent with the finding on stability of the red pigment from mulberry fruit (Xu *et al.* 2002).

Results in this study showed that high temperature had an influence on the stability of pansy pigment (Table 3). When the temperature was lower than 80°C, the pigment solution showed slight colour variation. However, when the temperature rose up to 100°C, the absorbance of pigment solution at 236 nm increased greatly, though the pigment colour remained light-red (Table 3). These results are in agreement with the pigment stability from black currant berries (Rubinskiene *et al.* 2005) and Moroccan cactus pear (EI Gharras *et al.* 2008), verifying increased degradation of the pigments subjected to high temperatures (80-100°C). Bordignon-Luiz *et al.* (2007) stated that light could induce degradation or oxidation of the natural pigment to lose original colour. In the present study, the colour of the pigment-water solution showed slight shifts under indoor natural light (140 lx) or UV (1000 lx) conditions for 6 hrs, compared with the controls (under the dark). While the colour of pigment solution changed from violet red to pink and showed a sharp increase in absorbance value in high-intensity outdoor sunlight (17000 lx) (Table 4). Result is consistent with the research realized by Xu *et al.* (2002) who verified that strong light significantly reduced the colour of anthocyanins from mulberry fruit.

Table 1. The solubility of pansy pigments in different solvents.

Solvent	Water	Methanol	95% alcohol	n-butyl alcohol	Acetone	Ethyl acetate	$CHCl_3$	CCl_4	Petroleum ether	0.1M HCl	0.1M NaOH
Dissolution	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble	Insoluble	Insoluble	Insoluble	Soluble	Soluble

Table 2. The absorbance and colour changes of pansy pigments extract at different pH.

pH	Control	1	2	3	4	5	6	7	8	9	10	11	12
Absorbance	4.28d[z]	4.19d	4.28d	4.48dc	4.73c	5.11bc	5.18bc	5.15bc	5.27b	5.36b	5.34b	c5.53a	5.73a
Color	violet red	violet red	violet red	violet red	Pale violet red	Pale violet red	Grey green	Grey green	Dark yellow	Dark yellow	Orange yellow	Orange yellow	Orange yellow

[z]Any two means within a column not followed by the same letter are significantly different at $p \leq 0.05$ by Tukey's *post hoc* test. The followings were as the same.

Table 3. The effect of temperature on the stability of pansy pigment.

T/℃	Control 18°C	25°C	40°C	60°C	80°C	100°C
Absorbance	4.925b[z]	4.821b	4.985b	4.845b	4.891b	5.257a
color	Violet red	Violet red	Orchid	Light red	Light red	Light red

Table 4. The effect of light intensity on the stability of pansy pigment.

Light condition	Colour	Light intensity	absorbance
Control (dark)	Violet red	0	4.325b[z]
Indoor natural light	Violet red	140 lx	3.984b
Ultraviolet treatment (UV)	Violet red	1000 lx	3.923b
Strong sunlight	pink	17000 lx	5.758a

Table 5. The effect of metal cations on the stability of pansy pigment.

Metal cations	Control (CK)	Na^+	Ca^{2+}	Mg^{2+}	Cu^{2+}
Absorbance	2.959b[z]	2.890b	2.795b	2.634b	5.455a
Colour	Orchid	Orchid	Orchid	Orchid	Turquoise

Co-pigmentation is a common effect between pigment and other colourless organic compounds, or metallic ions. Transformation and formation of the chemical bonds lead to the production of new molecular complex and then enhanced the colour of pigment (Eiro and Heinonen 2002, Gris *et al*. 2007, Castaneda-ovando *et al.* 2009). The present study showed that the influence of metal cations on the stability of pansy pigment varied with the type of metallic ions (Table 5). Na^+, Ca^{2+} and Mg^{2+} almost had no effects on the light absorbance and colour of pansy pigment solutions, which are in agreement with the study on stability of red pigment from mulberry fruit (Xu *et al*. 2002). However, the Cu^{2+} had an obvious influence on stability of pansy pigment with a sharp increase of the light absorbance intensity and the obvious colour change from orchid to turquoise. This observation is inconsistent with the research results on mulberry pigments, which indicated Cu^{2+} had no effect on the colour of these pigments (Xu *et al.* 2002). This difference may originate from the specific properties of different plants.

Pigment extract from pansy flower was easily dissolved in strong polarity solvents. It was sensitive to pH, UV and Cu^{2+} and moderately resistant to high temperature. Increasing the environmental factors like pH, temperature and light accelerates destruction of pansy pigments. So pansy pigment was more stable at pH 1.0 - 3.0, temperature lower than 80°C and weak light condition. Moreover, Cu^{2+} should be avoided in pansy pigment extraction process.

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