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# Effect of pH, temperature and iron on the stability of anthocyanins from black-skinned peanuts (*Arachis hypogaea* L.)

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**As public concerns about the safety of synthetic colorants increases, natural colors are assuming greater importance in the food and beverage industries. Anthocyanins are non poisonous abundant natural pigments. However, color deterioration during storage and processing limits their application as commercial colorants. The pigment extracts from black peanut skin are natural and safe colorants. The goal of this study was to study the effect of pH, temperature, metal ions, and ascorbic acid on the stability of pigments from the skins of black peanuts. The addition of Iron ($Fe^{3+}$) rapidly generated black or dark brown precipitate, but other metal ions did not significantly affect the absorbance of anthocyanins. The ideal conditions for extraction of anthocyanins from black peanut skins were pH 2.0 and 60°C.**

**Key words:** Black peanut, pre-extraction coloring, anthocyanin, stability, extrusion.

## INTRODUCTION

Color is one of the most important characteristics of fruit and vegetable products, and it could significantly affect customer's food and beverage selections (Stintzing and Carle, 2004). With increasing public concern about the safety of synthetic colorants, natural colors are assuming greater prominence (Malien-Aubert et al., 2001). Anthocyanins (glycosylated polyhydroxy derivatives of 2-phenylbenzopyrylium salts) are natural, water-soluble, non-toxic pigments responsible for some of the colors found in fruits, vegetables, flowers and other plant tissues (Mazza and Brouillard, 1990). Several studies have also focused on the application of anthocyanins in cancer treatments (Lule and Xia, 2005), their role in human nutrition (Stintzing and Carle, 2004), and their biological activity (Kong et al., 2003).

Anthocyanins have not been broadly used in foods and beverages, although they have been reported to be safe for use in dietary supplements (Bride and Timberlake, 1997). Their susceptibility to color deterioration during processing and storage has limited their application as commercial colorants (Cabrita et al., 2000; Malien-Aubert et al., 2001). In fact, much attention has been given to the intrinsic and extrinsic factors affecting pigment stability and color, such as temperature, the presence of light and pH value (Baranac et al., 1996; Bakowska et al., 2003; Parisa et al., 2007). Thermal processes including pasteurization, sterilization or concentration have been implicated in anthocyanin degradation and color loss (Sadilova et al., 2009). Thus the purpose of this study was to analyze anthocyanin stability in extracts of

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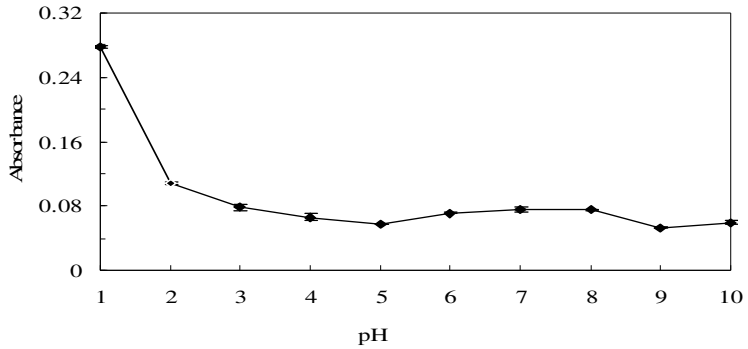


**Figure 1.** Effect of pH value (1-10) on the absorbance of anthocyanins.

pigment from black peanut skins by investigating the effects of parameters such as pH, temperature, and that of the addition of metal ions. The aim of the study was to identify suitable conditions for the extraction and storage of anthocyanins from this plant species for further detailed biochemical studies.

## MATERIALS AND METHODS

The black peanut (*Arachis hypogaea* L. heifeng 1) used for this study was purchased from Xinnong Agricultural Company, Hnandan, Hebei Province, China.

### Extraction of black peanut skin pigments

The dried and powdered black peanut skin pigment (1,000 mg) was mixed with ethanol (60%, v/v; 50 ml) containing 0.1% HCl solution and repeatedly extracted in a water bath at 60°C for 80 min. Fractions were filtered, and the solvents were concentrated under reduced pressure with a rotary evaporator, keeping the temperature of the bath at 40°C.

### Determination of effect of pH on stability of black peanut skin pigments

The extraction was carried out at pHs ranging from 1 to 10, and the absorbance of the filtered extracts was then measured at 525 nm for three replicates (Wang and Xu, 2007).

### Determination of effect of temperature on stability of black peanut skin pigments

The filtered extracts were placed in glass tubes with screw caps and incubated in a water bath at 30, 40, 50, 60, 70, and 80°C for 2 h. The absorbance of the filtered extracts was then measured at the given pH for three replicates (Wang and Xu, 2007).

### Determination of effect of metal ions on stability of black peanut skin pigments

The filtered extracts were placed in glass tubes and shaken with $Cu^{2+}$, $Mn^{2+}$, $K^{+}$, $Al^{3+}$, $Fe^{3+}$, $Zn^{2+}$, and $Mg^{2+}$ for 1 h. Color stability was measured by direct spectrophotometry without pH adjustment, and thus the obtained at 525 nm corresponded only to anthocyanins present in the cation form at the given pH (Wang and Xu, 2007).

### Determination of effect of ascorbic acid on stability of black peanut skin pigments

The filtered extracts were shaken in glass tubes and mixed with 0.01, 0.05, 0.1, 0.2 and 0.5% ascorbic acid for 0, 0.5, 1, 3, 20 and 24 h. The anthocyanin solutions with various levels of ascorbic acid were incubated in the dark for 1 h, and the level of anthocyanin absorbance at 525 nm was measured. All the spectrophotometric measurements were performed using a UV-Vis spectrophotometer (Prim, Secom am, France), using 1 cm path length glass cuvettes. After 1 h of equilibration at room temperature, anthocyanin absorbance at 525 nm was recorded (Marcovic et al., 2000).

### Statistical analysis

One-way analysis of variance (ANOVA) was performed using SPSS statistical analysis version 12.5 (SPSS Inc., Chicago, USA). Means for each parameter were compared by Tukey's test at a = 0.05. All measurements were carried out in three replicates, and each experiment was repeated three times.

## RESULTS AND DISCUSSION

### Effect of pH on the color stability of black peanut skin pigments

The absorbance of black peanut skin pigment (BPSP) varied over the pH range from 1 to 10, decreasing with increasing pH (2-10). To determine the effect of pH on the efficiency of total anthocyanin extraction, the pH of the extraction solution was adjusted to values ranging from 0.5 to 3.0 (Figure 1). The pH affected the amount of anthocyanin extracted. At pHs lower than pH 2.0, anthocyanins are present primarily in the form of red flavylium cations. When pH increases, the flavylium cations are converted into purple quinonoidal bases (Janna et al., 2007). These compounds are labile, and upon nucleophilic attack by water transform into colorless

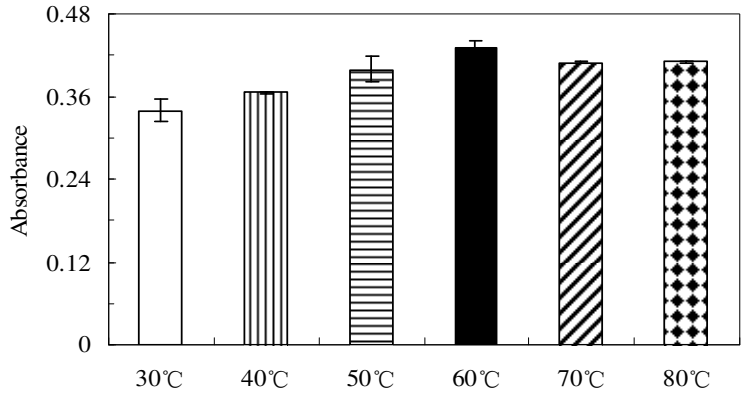


**Figure 2.** Effect of temperature on the absorbance of anthocyanins.

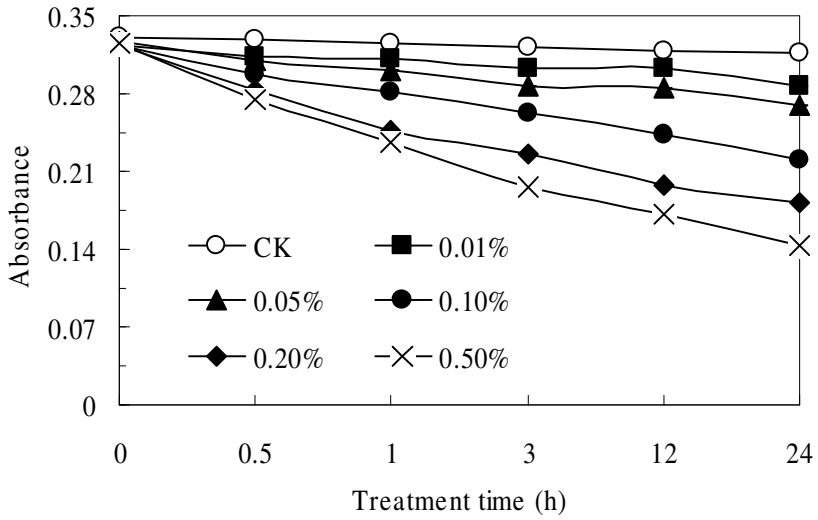


**Figure 3.** Effect of ascorbic acid on the absorbance of anthocyanins.

carbinol pseudobases and chalcone pseudobases. Dao et al. (1998) reported that the same anthocyanin may have different colors in different plants, depending on the pH of the organelle.

## Effect of temperature on the stability of black peanut skin pigments

The absorbance of BPSP increased with increasing temperature, reaching the highest levels at 60℃ (Figure 2), then decreased at higher temperatures. Bakowska et al. (2003) reported similar results for the effect of temperature on copigmentation. In addition, these results are similar to the previous studies (Kucharska et al. 1998; Mazza and Miniati, 1993). Marcovic et al. (2000) reported that temperature was a significant factor in the thermodynamic conditions of the copigmentation progress.

## Effect of ascorbic acid on the stability of black peanut skin pigment

The absorbance of BPSP was decreased with the concentration of ascorbic acid, and also decreased with time (Figure 3). The absorbance of BPSP was decreased by 54.9% with 0.5% ascorbic acid after 24 h treatment. Kalt et al. (1999) reported that ascorbate content and antioxidant capacity were negatively correlated (R = -0.80) since ascorbate levels were low in the fruit where antioxidant capacity was high.

## Effect of metal ions on the stability of black peanut skin pigment

The absorbance of BPSP was affected by the addition of metal ions (Table 1). The absorbance of peanut skin pigment anthocyanin was higher with the addition of $Cu^{2+}$

**Table 1.** Effect of metal ions on the absorbance of peanut skin pigments.

| Metal ions | Control | $Cu^{2+}$ | $Mn^{2+}$ | $K^{+}$ | $Al^{3+}$ | $Zn^{2+}$ | $Mg^{2+}$ |
|---|---|---|---|---|---|---|---|
| Absorbance (525 nm) | 0.324 | 0.427 | 0.307 | 0.311 | 0.514 | 0.321 | 0.312 |

and $A1^{3+}$. Absorbance decreased with the addition of $Mn^{2+}$ and $Mg^{2+}$, but it was not significantly affected by $K^{+}$ and $Zn^{2+}$. $Fe^{3+}$ rapidly generated a black-dark brown precipitate.

## Conclusions

The color, intensity and stability of the black-skinned peanut anthocyanin varied significantly in the pH range tested (pH 1 to 10). Anthocyanin stability and color intensity stabilized at more neutral pHs (pH 5 to 7), accompanied by a gradual bathochromic shift to more bluish colors. Other factors not directly addressed in this study (e.g., buffer composition, concentration) may influence the pH-dependency of the color and stability of pure anthocyanins in aqueous media.

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