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*Full Length Research Paper*

# Investigation of the stability and antioxidant properties of anthocyanins-based purple potato colorants after processing

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**To investigate the effect of processing method on the stability and antioxidant properties of anthocyanins-based colorants, purple potato (*Solanum tuberosum* L.) was treated by steaming, baking before lyophilization, oven drying and direct lyophilization before extracted by 0.5% HCl-methanol solution twice. The concentration of total anthocyanins in different treated samples ranged from 0.34 to 1.03 mg/g fresh weight. The direct lyophilization treated sample had significant higher content than other processing method. Stability study showed that both light and heat could accelerate the degradation of anthocyanin-based purple potato colorant. The fresh purple potato colorant showed the most stable property, followed by the lyophilization, oven drying, steaming before lyophilization and baking before lyophilization samples. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability of purple potato extracts ranged from 0.25 to 0.37 mg/ml. The ability of the extracts to reduce $Fe^{3+}$ to $Fe^{2+}$ ranged from 395.40 to 1320.47 µmol/L equivalent to $FeSO_4$ at the concentration of 10 mg/ml. Lyophilization treated samples showed the most strongest antioxidant activity, significantly higher than that of other processing methods. Our results suggest that to keep stable and functional anthocyanins-based purple potato colorants, lyophilization was a recommended suitable processing method in food industry.**

**Key words:** *Solanum tuberosum* L., processing methods, anthocyanin-based colorant, stability, antioxidant.

## INTRODUCTION

Due to the growing interest of substituting synthetic colorants with toxic effects and carcinogenicity potential in humans, the study of natural colorants is becoming an extensive and active area of investigation (Chou et al., 2007). The natural colorants show some beneficial health effects and safety. Anthocyanins (Figure 1), as one of the natural pigments, play an important role as antioxidant, anti-platelet aggregation and anti-inflammatory (Manach et al., 2005; Prior and Wu, 2006). The structures of six commonly used anthocyanidins in food industry are listed in Table 1. Anthocyanins not only scavenge free radicals, bind heavy metal such as iron, zinc and copper, but also are inducers of antioxidant enzymes such as glutathione-S-transferase (GST) and superoxide dismutase (SOD) (Li et al., 2005; Duthie et al., 2006; Manach et al., 2005).

Purple potato is a variety of potato (*Solanum tuberosum* L.), which possesses purple peer and purple pulp. It contains abundant carbohydrates, vitamins, minerals, proteins and amino acids. In addition, they are rich in various forms of anthocyanins, including petunin, peonin and their derivatives of caffeic acid (Torgils et al., 2003). Anthocyanins have a high potential as natural colorants due to their attractive orange, red, purple, and blue colors. However, they have stability problems (Markakis, 1982; Francis, 1982). The color and stability of anthocyanin pigments are dependent on several factors, including structure and concentration of the pigment, pH, temperature, light intensity and quality, presence of co-pigments, metallic ions, enzymes, oxygen, ascorbic acid,

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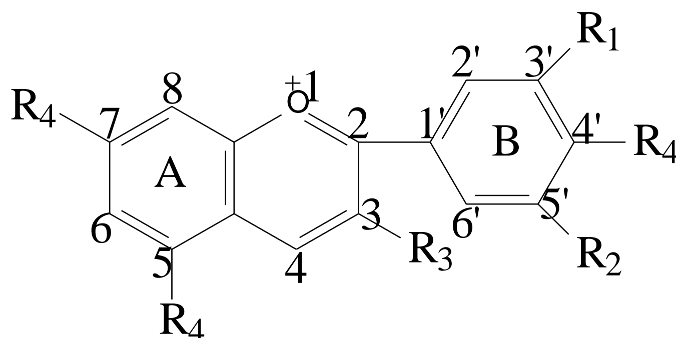


**Figure 1.** Basic structure of anthocyanins. $R_1$, $R_2$ represent H, OH or $OCH_3$; $R_3$ - saccharide groups or H; $R_4$ - saccharide groups or OH.

**Table 1.** Structure of six common used anthocyanidins in food industry.

| Anthocyanidins | Substituent group | | | | | | | Proportion of natural total anthocyanidins (%) |
|---|---|---|---|---|---|---|---|---|
| | 3 | 5 | 6 | 7 | 3' | 4' | 5' | |
| Cyanidin (Cy) | OH | OH | H | OH | OH | OH | H | 31 |
| Pelargonidin (Pg) | OH | OH | H | OH | H | OH | H | 18 |
| Delphinidin (Dp) | OH | OH | H | OH | OH | OH | OH | 15 |
| Peonidin (Pn) | OH | OH | H | OH | OMe | OH | H | 11 |
| Malvidin (Mv) | OH | OH | H | OH | OMe | OH | OMe | 10 |
| Petunidin (Pt) | OH | OH | H | OH | OMe | OH | OH | 9 |

sugars and their degradation products and sulfur dioxide, among others (Mazza and Miniati, 1993; Francis, 1982).

In acidic media, four anthocyanin structures exist in equilibrium: Flavylium cation, quinonoidal base, carbinol pseudobase and chalcone (Figure 2). The relative amounts of these structures at equilibrium vary with pH and anthocyanin structure (Brouillard, 1988; Mazza and Miniati, 1993). Thus, studies on the purple potato are focus on the extraction technology and stability of the purple colorant. Furthermore, what is easily ignored in current studies is that humans consume purple potato usually after a serious processing, such as steaming, baking, drying and so on. Processing would severely affect the chemical composition and properties of the colorant. This study compared the anthocyanin content, stability and antioxidant property of the colorant extracted from the purple potato after being treated by steaming before lyophilization, baking before lyophilization, over drying and direct lyophilization, with the aim to seek for a suitable processing method to exploit anthocyanin-based purple potatoes in a more scientifically and efficiently way.

## MATERIALS AND METHODS

Purple potatoes were cultivated at the experimental farm of Zhejiang Agriculture and Forestry University, Linan, Zhejiang, China.

### Sample preparation

Fresh purple potatoes were collected in autumn season in Linan district, 2009, and then were washed with distilled water and dried thoroughly. These purple potatoes were kept together during storage, but were randomized for later processing. The purple potatoes were divided into four groups and then treated in four different ways (Table 2). All of the dried samples from four treatments were collected, weighted, smashed, and were ready for extraction.

### Extraction

The extraction of colorant was accomplished according to a

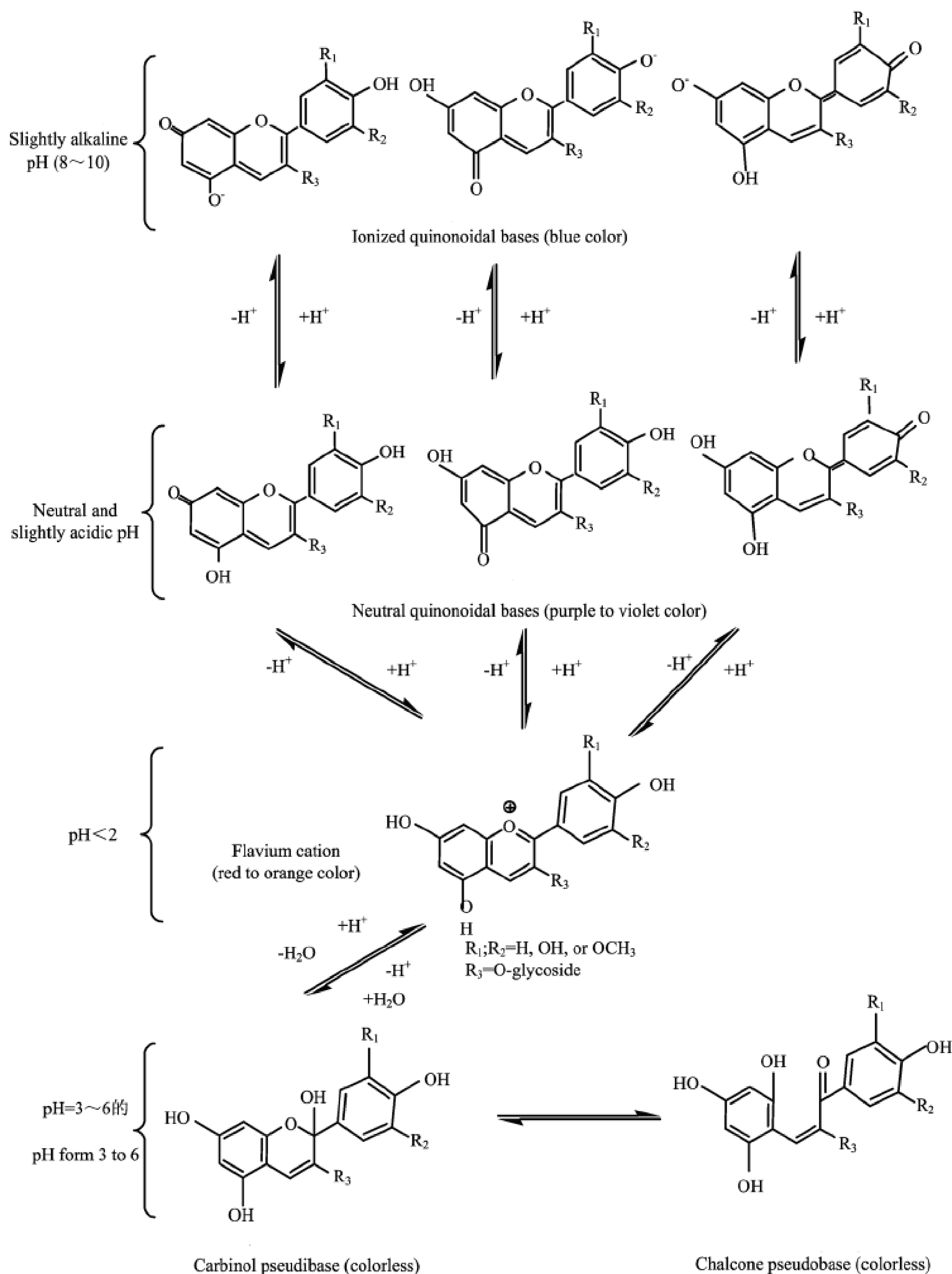


**Figure 2.** The molecular conformation of anthocyanins in different pH solution at room temperature.

modification of the methods reported in the literature (Mazza et al., 2004; Naczk and Shahidi, 2006).

Briefly, 100 ml 0.5% HCl methanol was added to a flask containing 5 g ground purple potato powder. After refluxing for 2 h twice, the extract was filtered and evaporated to dryness at 40°C and reconstituted in methanol to 50 ml. Each methanol solution of sample was spectro prescaned from 400 to 600 nm. The maximum absorption wavelength of each colorant solution was 535 to 536

**Table 2.** Processing method of different treatment groups.

| Group | Processing method |
|---|---|
| 1 | 100 g of purple potato was cut into 1 mm slices and then lyophilized. |
| 2 | 100 g of purple potato was cut into 1 mm slices and then dried in the oven at 50° until counter weights |
| 3 | 100 g of purple potato was cut into 1 mm slices and then steamed in the electric cooker. The steamed samples were then lyophilized. |
| 4 | 100 g of purple potato was cut into 1 mm slices and then roasted in microwave oven. The roasted samples were then lyophilized. |

nm.

## Experimental procedures

### Total anthocyanins measurement using pH differential method

Total anthocyanins were measured according to a modification of the methods described earlier (Fuleki and Francis, 1968; Giusti and Rodriguez, 1999; Farah et al., 2008). Two dilutions of the sample were prepared. For pH 1.0 buffer, 1.49 g KCl was dissolved into 100 ml deionized water, and then carefully poured 1.7 ml concentrated HCl into 100 ml deionized water of 0.2 N. Furthermore, 25 ml of the KCl solution was mixed with 67 ml of the 0.2 N HCl solutions. The pH was adjusted to 1.0 ± 0.1 if necessary. While for the pH 4.5 buffer, 1.64 g sodium acetate was dissolved in 100 ml deionized water and adjusted to pH 4.5 ± 0.1 with HCl.

To measure the anthocyanin content, first, a 1.0 ml aliquot of the purple potato solution was removed and placed into a 25 ml volumetric flask. This was then diluted to volume with pH 1.0 buffer and mixed. Secondly, 1.0 ml aliquot of the purple potato solution was removed and placed into a 25 ml volumetric flask. This was then diluted to volume with pH 4.5 buffer and mixed. The absorbances of the pH 1.0 and pH 4.5 sample preparations were measured at 510 and 700 nm, respectively. Distilled water was used as blank. The concentration (% w/w) of each anthocyanin in the sample was calculated according to the following formula and expressed as cyanidin-3-glc equivalents:

Absorbance = ($A_{510\ nm}$ pH 1.0 - $A_{700nm}$ pH 1.0) - ($A_{510nm}$ pH 4.5 - $A_{700nm}$ pH 4.5)

The percent weight per weight (%w/w) of total anthocyanins in the sample was calculated as:

$$\%w/w = \frac{A}{\varepsilon L} \times MW \times DF \times \frac{V}{Wt} \times 100\%$$

Where, A is the absorbance; ε is the extinction coefficient (26,900 for cy-3-glc); MW is the molecular weight (449.2 g/mol for cy-3-glc); DF is the dilution factor (1 ml sample is diluted to 25 ml, DF = 25); V is the final volume (50 ml); Wt is the sample weight (5000 mg); L is the cell path length (1 cm). For comparison, the same extinction coefficient was used for other standards to calculate the concentration of each anthocyanin and thus results reported was expressed as Cy-3-glc equivalents.

### Stability analysis

Each methanol solution of colorant was used for anthocyanin-based colorant stability studies. Light effect on colorant stability was performed with samples inside capped glass vials sealed with parafilm and exposed to outside sunlight, inside natural light, and protected from the light, respectively. The absorbance was measured every three days (Rubinskiene et al., 2005). To determine the impact of temperature and thermal treatment time on the stability of colorant. Samples were placed in capped glass vials and heated at 40, 60, 80 and 90°C in a water thermostat. The absorbance was measured every hour. The results were expressed as colorant survival rate calculated as follows:

Survival rate (%) = $A/A_0 \times 100$

Where, A is the absorbance of test compounds after a period of time,and $A_0$ is the absorbance of the initial test compounds.

### DPPH scavenging assay

The hydrogen atom of electron donation abilities of the corresponding colorant were measured from the bleaching of the purple-colored methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The method of Brand et al. (1995) with slight modifications was used for measuring the DPPH radical scavenging ability of the colorant (Lim and Quah, 2007). DPPH was dissolved in methanol at a final concentration of 0.101 mmol/L. An aliquot of methanolic solution of sample colorant extracts (0.1 ml) at different concentrations were added to 3.9 ml DPPH solution. Four different treated purple potato colorant extracts were used for each assay. After 1 h incubation period at room temperature, the absorbance was measured against a blank (containing all reagents except the test compound) at 510 nm. Ascorbic acid solution was used as the positive control. Triplicate tubes were prepared for each extract. The results were expressed as % DPPH radical scavenging rate calculated as follows:

Radical scavenging rate (%) = $(A_{blank} - A_{sample}) / A_{blank} \times 100$

Where, $A_{blank}$ is the absorbance of the control reaction (containing all reagents except the test compound), and $A_{sample}$ is the absorbance of the test compounds. $IC_{50}$ is the amount of antioxidant necessary to decrease the initial concentration of DPPH radical (0.10 mmol/L) by 50%.

### Determination of reducing power using ferric reducing ability of plasma (FRAP) assay

This method was proposed by Oyaizu (1986), which involves the presence of antioxidants in an extract to reduce the ferricyanide complex to the ferrous form with some modification (Benzie and Strains, 1996; Amin and Razieh, 2007). Briefly, the FRAP reagent containing 2.5 ml of 10 mmol/L tripyridyltriazine (TPTZ) solution in 40 mmol/L HCl plus 2.5 ml of 20 mmol/L $FeCl_3$ and 25 ml of 0.3 mol/L acetate buffer, pH 3.6, was freshly prepared. The extracts

**Table 3.** Total anthocyanin contents (mg/g fresh weight) in the different treated purple potatoes.

| Different treated sample | Oven drying | Steaming before lyophilization | Lyophilization | Baking before lyophilization |
|---|---|---|---|---|
| Content (mg/g fresh weight) | 0.58 ± 0.01[b] | 0.36 ± 0.00[a] | 1.03 ± 0.01[c] | 0.34 ± 0.01[a] |

[Abcd]Means values in the same column with different letters are significantly different: ($\rho$<0.05).

were diluted in methanol at three different concentrations. An aliquot of 0.3 ml of the solution was mixed with 2.7 ml of FRAP reagent. The absorption of the reaction mixture was measured at 593 nm. A volume of 0.3 ml methanol and 2.7 ml TPTZ reagent mixture was used as blank. Aqueous solutions of known Fe (II) concentration, in the range of 0 to 1000 µmol/L ($FeSO_4$) were used for obtaining the calibration curve. Triplicate tubes were prepared for each extract. The reducing power of sample was expressed as equivalent concentration of $FeSO_4$. This FRAP parameter was defined as the concentration of antioxidant having a ferric reducing ability equivalent to that of 1 µmol $FeSO_4$.

#### Statistical analysis

The determination of anthocyanin contents and the DPPH radical scavenging ability of different treated purple potato extracts were carried out in triplicate and the results were given as mean ± standard deviation (S.D). Data were analysed by one-way analysis of variance (ANOVA). Significant differences were assessed with as LSD test ($\rho$< 0.05). The statistical analysis was performed using SPSS (version 16.0; SPSS Inc., Chicago).

## RESULTS

### Anthocyanin composition using pH differential method

Anthocyanin pigments undergo reversible structural transformations with a change in pH manifested by strikingly different absorbance spectra. The oxonium form predominates at pH 1.0 and the hemiketal (colorless) form at pH 4.5. The pH-differential method is based on this reaction and permits accurate and rapid measurements for the total amount of anthocyanins, even in the presence of polymerized degraded pigment and other interfering compounds (Fuleki and Francis, 1968; Farah et al., 2008). Since Cy-3-glc was the most abundant anthocyanin, the extinction coefficient of Cy-3-glc standard was used to measure the total anthocyanins.

Significant differences in the anthocyanin content were found between differently treated purple potato extracts. Table 3 shows the total concentration of the anthocyanins (mg/g fresh weight) found in the different treated samples. The concentration of total anthocyanins in different treated samples ranged from 0.34 to 1.03 mg/g fresh weight. Purple potato with lyophilization presented the most total anthocyanins and with significant difference from the other samples. Samples treated by steaming and baking were the varieties with lower anthocyanin content, and without significant differences between them. Thus, the purple potato possessing abundant anthocyanin is a good material for natural colorant exploitation. And the treatment with low temperature to the material was essential for keeping anthocyanin.

### Effect of light on different treated purple potato colorant extracts stability

The effect of light on colorant stability was performed with exposing samples to outside sunlight, inside natural light, and protected from the light, respectively. The colorant survival rate of different treated purple potato, including fresh purple potato colorant extract, at each light condition is shown in Figure 3A to C. The result suggests that light could accelerate the degradation of anthocyanin-based purple potato colorant. After 18 days incubation period under sunlight, the survival rate of the different treated purple potato shifted from 50.80 to 64.1%, but still kept a rate of 66.70 to 82.90% if the samples were prevented from the light. Thus, the anthocyanin-based colorant of purple potato should be kept away from light during storage.

Cooking, including steaming and baking may result in a loss of some compounds and reform some new compounds (Sahilin et al., 2004). From our results, the composition of colorant in purple potato changed and consequently became less stable with cooking than without cooking. Under the all light condition (sunlight, natural light and keep from light), the fresh purple potato colorant showed the most stable property, then the lyophilization sample, oven drying, steaming before lyophilization and baking before lyophilization at last. The results therefore suggested that cooking may destroy the stability of the purple potato colorant.

### Effect of temperature on stability of different treated purple potato colorant extracts

The thermal stability of anthocyanin-based purple potato colorant with different process was studied at 40, 60, 80 and 90°C. The results (Figure 4A to D) show that thermal treatment has a significant effect on the degradation of colorant. Thermal degradation was slow at a low temperature, but the rate accelerated as the temperature increased. As for the fresh, oven drying and lyophilization samples, the colorant was comparatively stable when the temperature was below 60°C, and degraded dramatically when it reached 80°C or above. Moreover, colorant from the steaming and baking treated sample was much more

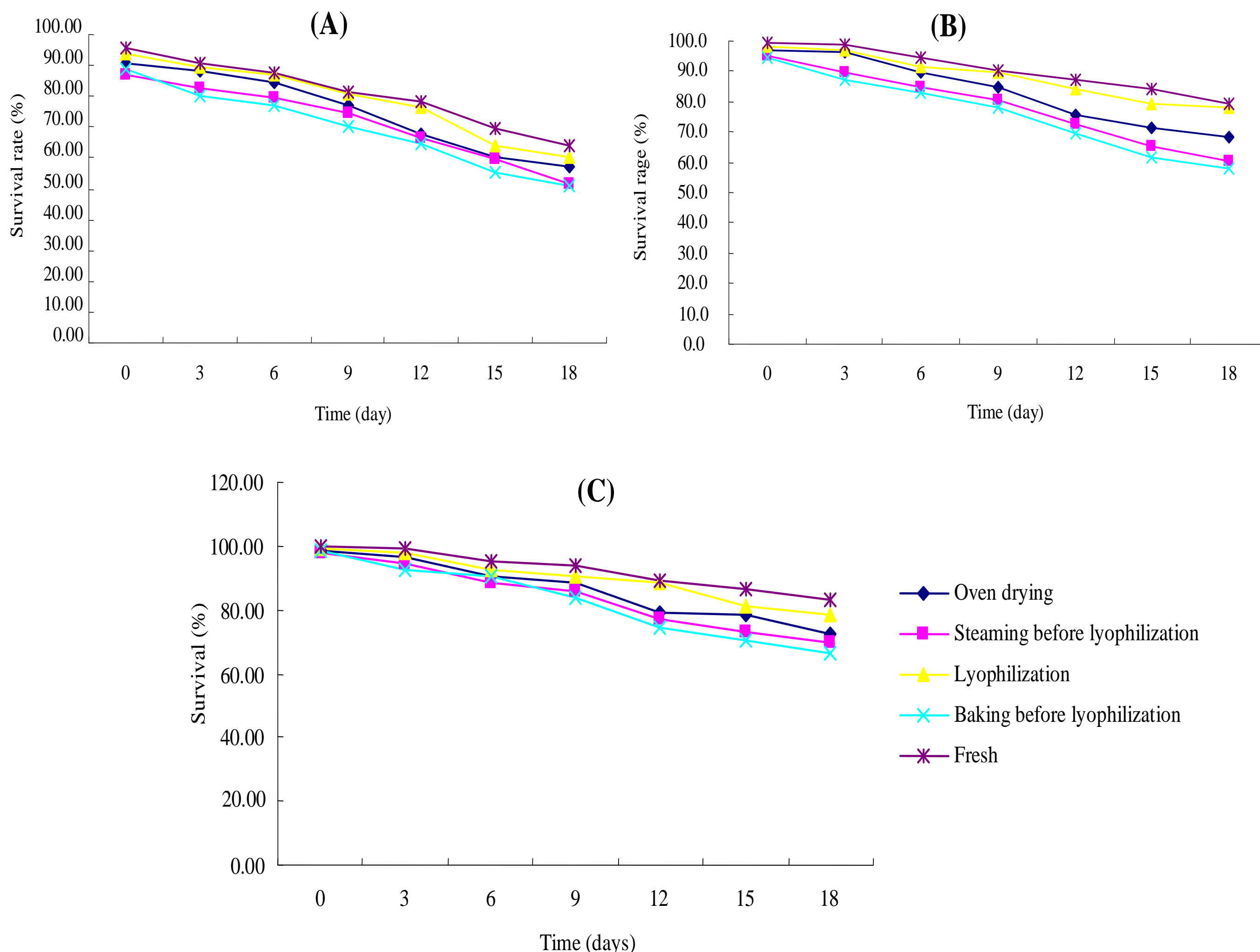


**Figure 3**. Effect of different processing method on the stability of the anthocyanins- based purple potato colorant in different light treatment. (A) Exposed to the outside sunlight; (B) inside natural light; (C) protected from light.

sensitive to temperature. This may be because of the heat absorption of the ring-opening chromene. Thus, the anthocyanin-based colorant from the purple potato should be kept away from long time heating during processing and usage.Meanwhile, under different temperatures (40, 60, 80 and 90°C), the steaming and baking treated samples showed more sensitivity to temperature, which was different from the sample without heat treatment. When the temperature was 80°C, the oven drying sample showed similar thermal sensitivity as the above two samples after incubation for 4 h. Thus, heat treatment would lower the stability of purple potato colorant.

## DPPH radical scavenging ability

The scavenging ability of 1,1-diphenylpicrylhydrazyl (DPPH) radical by various purple potato colorants was evaluated. The assays were carried out in methanol and the results expressed as $IC_{50}$, which represents the antioxidant concentration necessary to scavenge the initial DPPH concentration by 50%. We determined the DPPH radical scavenging rate of different treated purple potato extracts at different concentrations. Vitamin C solution was used as positive control for comparing the antioxidant potential. The $IC_{50}$ value of the each sample was reported in Table 4. Lower values correspond to higher radical scavenging capacity. The DPPH radical scavenging ability of purple potato extracts was significantly lower than that of Vit. C solution, ranging from 0.25 to 0.37mg/ml. Baking may destroy the antioxidant compounds in the purple potato, hence the sample with baking treatment showed the lowest DPPH radical scavenging ability. However, the sample with steaming possessed similar anthocyanins content with that of baking as it showed a significantly different DPPH radical scavenging ability. This mechanism, however, needs further investigation.

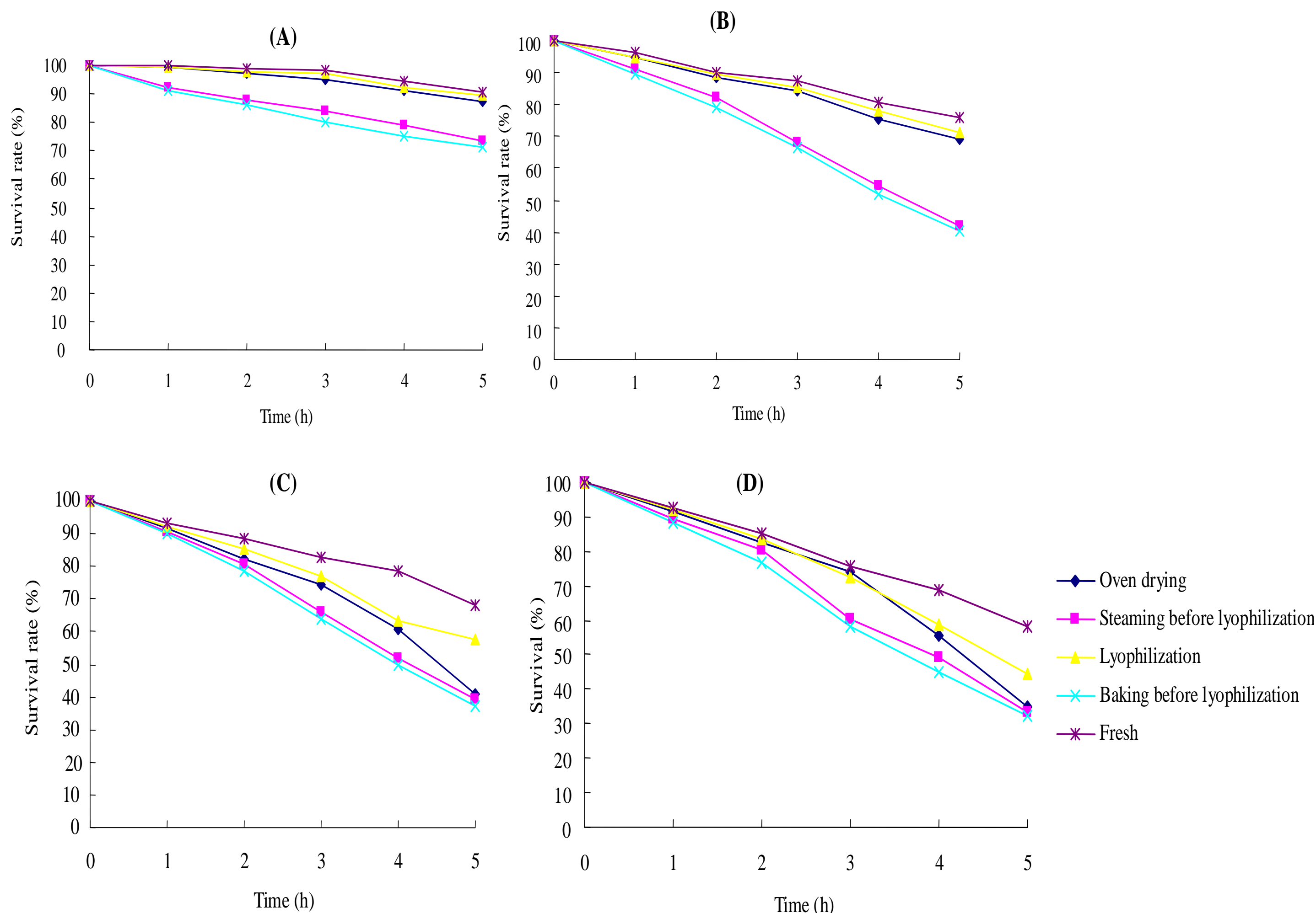


**Figure 4.** The degradation of the anthocyanins-based colorant from the different treated purple potato at different temperature. (A)40°C; (B) 60°C; (C)80°C; (D)90°C.

**Table 4.** Inhibition concentration ($IC_{50}$) of different samples with DPPH assay.

| Different treated samples | Oven drying | Steaming before lyophilization | Lyophilization | Baking before lyophilization | Vitamin solution |
|---|---|---|---|---|---|
| $IC_{50}$(mg/ml) | 0.35 ± 0.02$^c$ | 0.34 ± 0.01$^c$ | 0.25 ± 0.04$^b$ | 0.37 ± 0.05$^d$ | 0.01 ± 0.00$^a$ |

$^{abcd}$Means values in the same column with different letters are significantly different: (ρ<0.05).

## Reducing power

FRAP assay measures the change in absorbance at 593 nm due to the formation of a blue colored $Fe^{2+}$-tripyridyltriazine compound from colorless oxidized $Fe^{3+}$ form by the action of electron donating antioxidants. The ability of different treated purple potato extracts to reduce $Fe^{3+}$ to $Fe^{2+}$ ranged from 395.40 ± 26.32 to 1320.47 ± 27.78 µmol/L at a concentration of 10 mg/ml (Table 5). The lyophilization sample showed the strongest reducing power, which was significantly higher than that of other treated samples. This was followed by samples treated by baking before lyophilization and oven drying. However, steaming before lyophilization treated sample demonstrated the lowest reducing power.

## DISCUSSION

Free radicals (or reactive oxygen species), as ions, atoms or molecules with unpaired electrons, can occur in a chain reaction with almost any inert substances under

**Table 5.** FRAP value of different samples with FRAP assay (μmol/L)

| Concentrations (mg/ml) | *Oven drying | Steaming before lyophilization | Lyophilization | Baking before lyophilization |
|---|---|---|---|---|
| 10 | 679.71 ± 29.00[b] | 395.40 ± 26.32[a] | 1320.47 ± 27.78[d] | 812 ± 17.80[c] |

[abcd]Means values in the same column with different letters are significantly different: (ρ<0.05). *Samples

any inert condition. Pharmacological studies have shown that many diseases of organisms such as aging, atherosclerosis, cancer, inflammation, etc. are related to the oxidative damage by the excess free radicals in the body (Martinez, 1995; Halliwell, 1996; Sandeep and Brian, 2000). Polyphenolic compounds, including anthocyanins that are widely distributed in many plants, have a very strong free radical scavenging capabilities. They can effectively clear the excess free radicals in the body (Francilene et al., 2011; Nuri et al., 2010; Chiunghui et al., 2010), thereby playing an important role in anti-aging and preventing many free radical related diseases.

Nowadays, the study of natural colorants is an extensive and active area of investigation due to the growing interest of substituting synthetic colorants with toxic effects in humans (Araceli et al., 2009). Anthocyanins are amongst the most utilized vegetable colorants in the food industry attributed to their safety and various biological activities, for example antioxidant, anti-inflammatory, anti-mutagenicity, antitumor, improving eyesight, prevention and treatment of cardiovascular diseases and neurological diseases (Konczak and Zhang, 2004). Thus, anthocyanins-based colorants have been widely used in food and medicine industry in several countries.

Purple potato (*S. tuberosum L.*), which possesses purple peer and purple pulp, are rich in polyphenolic compounds such as various forms of anthocyanins. It is a potential functional natural anthocyanin-based colorant resource for exploitation. However, anthocyanin-based colorants, including purple potato colorant, are usually highly unstable and very susceptible to degradation (Giusti and Wrolstad, 2003), thereby limiting their utilization.

Therefore, the anthocyanins chemical stability is the main focus of recent studies due to their abundant and potential applications, their beneficial effects and their use as alternative to artificial colorants (Rein, 2005). Until now, in the purple potato research field, what is easily ignored is that humans consume purple potato usually after a serious processing, such as steaming, baking, drying and so on. Processing would severely affect the chemical composition and properties of the colorant. Hence, in order to exploit purple potato colorant effectively, the processing method and storage condition need to be paid more attention.

Thus, the present study compared the anthocyanin-based colorant stability of the antioxidant activity of different processed purple potatoes. Our result illustrates that the fresh purple potato colorant showed much more stable property compared with the lyophilized, oven dried, steamed before lyophilization and baked before lyophilization treated samples. Both light and heat would accelerate the degradation of anthocyanin-based purple potato colorant.

Therefore, the anthocyanin-based colorant from the purple potato should be protected from long time heating and light during processing and usage. Meanwhile, the antioxidant activities of purple potato colorant illustrated by DPPH and FRAP assays showed high correlation with anthocyanins. Direct lyophilization treatment, which was without heat showed much stronger antioxidant activity than other processing method treated samples. The present results may provide a theoretical basis for scientifically exploitation of anthocyanins-based purple potato colorant. Also, the composition of the purple potato colorant with different processing method needs further investigation.

## Conclusion

Significant difference existed in the antioxidant capacity of four different processed purple potato (*S. tuberosum* L.) extracts assayed via DPPH and FRAP, as well as the total anthocyanins content and the anthocyanin-based colorant stability.

High temperature treatment would destroy the anthocyanin compounds and significantly decrease the anthocyanin-based purple potato colorants. Meanwhile, different antioxidant assays may attain different results of antioxidant abilities, thus different antioxidant assays were necessary to objectively estimate the natural antioxidants' abilities. Our results suggest that in order to exploit and utilize purple potato colorant more effectively, colorant should be kept away from light and heat treatment.

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