J. Cosmet. Sci., **61,** 333–341 (September/October 2010)

Influence of purple sweet potato extracts on the UV absorption properties of a cosmetic cream

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Accepted for publication June 30, 2010.

Synopsis

Anthocyanins were added to a cosmetic cream to provide additional protection against UV radiation. The influence of anthocyanins on UV absorption ability was carefully evaluated. Anthocyanins were successfully extracted from TNG73 purple sweet potato using acidic ethanol and acidic water. Acidic ethanol-extracted anthocyanins had better radical scavenging ability, higher total phenolic content, and stronger reducing ability than acidic water-extracted anthocyanins. The cosmetic cream with 0.61 mg of total anthocyanins (per 100 g cream) absorbed approximately 46% of the incident UV radiation. Although the anthocyanins absorbed both UV-A and UV-B radiation, they were particularly effective against UV-B rays. This study demonstrates that the addition of anthocyanin extracts of purple sweet potato to a cosmetic cream improves the cream's UV absorption ability.

INTRODUCTION

The sun's rays contain ultraviolet light that can be classified as UV-A (320~400 nm), UV-B (290~320 nm), or UV-C (200~290 nm) radiation. Ultraviolet rays can penetrate into skin tissues and destroy skin cells (1). As a result, ultraviolet rays cause a number of serious problems including visible aging, wrinkling of the skin, damage to the immune system, and skin cancer (2,3). The use of sunscreen products to protect skin from ultraviolet radiation is strongly recommended. The ability of sunscreen to protect against UV radiation is measured by two factors, the sun protection factor (SPF) and the protection factor of UV-A (PFA). The SPF number indicates the protection from sunburn caused by UV-B, while the PFA number indicates the protection against UV-A (4). In both cases, a higher number indicates stronger protection. Sunscreens work by chemical (absorbing) and/or physical (reflecting and scattering) methods to protect the skin from ultraviolet

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radiation. Some organic materials like octyl dimethyl para-aminobenzoic acid (PABA) and salicylate are used to absorb ultraviolet radiation, while some inorganic particles like zinc oxide and titanium dioxide are used to reflect the ultraviolet rays (5–8). Both chemical and physical methods use active ingredients to insulate the skin from ultraviolet radiation, but unfortunately some ingredients may act as allergens to the skin. In addition, the active ingredients may break down after hours of exposure to the sun. This decomposition raises questions about how long the UV protective ability of sunscreens will last. Consumers demand comfortable, stable, and safe sunscreen ingredients (9). The current trend in the sunscreen market is towards using ingredients extracted from natural products, which are generally considered safe (10–13).

Anthocyanins are good anti-oxidative compounds (14–16). Since anthocyanins have been reported to have the ability to absorb UV-A and UV-B radiation (17,18), they can also protect skin against wrinkling and other aging effects. Although anthocyanins are ideal ingredients for making sunscreens, it is important to maintain an acidic environment to avoid degradation of anthocyanins (19). Anthocyanins are pH-sensitive (20,21), and their color changes with pH. The effect of pH on the UV absorption ability of anthocyanins requires further study.

Anthocyanins can be found in many deep-colored vegetables and fruits such as grapes, cherries, plums, eggplants, and cauliflower (22–25). Anthocyanins and their derivatives are widely used as natural pigments and food additives. Crop scientists at the Agricultural Research Institute (ARI), Chia-Yi Agricultural Experiment Station, have been working on a sweet potato breeding program to create new cultivars. A newly cultivated purple sweet potato, named Tainung No. 73 (TNG73), has reddish-purple root flesh. TNG73 is the first sweet potato variety developed by a Taiwanese breeder with high anthocyanin content. It was proven that the anthocyanins extracted from purple sweet potato have increased stability during thermal treatments (26). Anthocyanins can be directly extracted from purple sweet potato using acidic ethanol and acidic water. Although new extraction methods have been developed, acidic-solvent extraction is still preferred due to its relatively low cost.

The main goal of this study was to incorporate TNG73 purple sweet potato extract into a cosmetic cream and analyze the influence of anthocyanins on the apparent UV protection. The DPPH radical scavenging activity, total phenolic content, and the reducing ability of the anthocyanin extracts were also carefully evaluated.

MATERIALS AND METHODS

TNG73 purple sweet potatoes provided by the Agricultural Research Institute (ARI), Chia-Yi Agricultural Experiment Station, Taiwan, were used as the anthocyanin source in this study. TNG73 was extracted with either acidic ethanol (1.5 N HCl in ethanol; 15:85 (v/v)) or acidic water (1.5 N HCl in water; 15:85 (v/v)) to provide anthocyanin solutions. The extracted anthocyanin solutions were purified using resins, then freeze-dried to collect the reddish-purple powders. The total anthocyanin content, expressed as cyanidin-3-glucoside, was determined by the following equation (27):

$$\text{Total anthocyanin content (mg/l)} = \frac{(A_{pH1.0} - A_{pH4.5}) \times MW \times DF \times 1000}{\varepsilon \times d} \quad (1)$$

where $A_{pH1.0}$ = measured absorbance in pH 1.0 potassium chloride buffer with HCl; $A_{pH4.5}$ = measured absorbance in pH 4.5 sodium acetate buffer with HCl; DF = the dilution factor; MW = the molecular weight of cyanidin-3-glucoside, 449.2; ε = molar absorptivity, 26,900; and d = the optical path of the cuvette (1 cm).

Based on the ingredients of commercial sunscreens collected from the market, a plain cosmetic cream recipe was designed for this study to test the UV-absorbing ability of purple sweet potato extract. The cosmetic cream contained 5% emulsifier, 5% Tween 80, 2% olive oil, 2% xanthan gum, 0.2% disodium EDTA, 1% sodium chloride, 0.2% methylparaben, 0.2% butylparaben, and distilled water. Then 5 or 10 ml of purple sweet potato extracts were added per 100 g of cream. The UV-visible spectra of anthocyanin extracts were analyzed using a Jasco^{R} V-530 UV/VIS spectrophotometer. Acidic ethanol solution (0.1N $HCl_{(aq)}$ in ethanol, 15:85 (v/v)), was used to dilute the samples before running spectrophotometric analysis. To study the influence of pH, 0.1 ml of anthocyanin extracted solution was dissolved in 40 ml of distilled water. The pH of the solution was increased from acidic to basic by slowly adding 0.1N $NaOH_{(aq)}$. Samples with pH ranging between 4 and 11 were used for UV absorption analysis.

The scavenging activity of anthocyanin extracts on DPPH (1,1-diphenyl- 2-picrylhydrazyl) radicals was determined using the method provided by Huang *et al.* (28). The radical scavenging activity of ascorbic acid was measured as a positive control. Fifty microliters of the extract was mixed with 150 µl of freshly prepared 1 mM DPPH in ethanol. The mixture was kept in the dark for 30 min. The absorbance of the mixture at 517 nm was then measured using an ELISA reader (TECAN^{R}, Austria). The radical scavenging activity was calculated as follows: $(1- (A_{Sample}/A_{Blank})) \times 100$. The EC_{50} value was defined as the effective concentration at which 50% of the DPPH radicals were scavenged, and was determined by interpolation based on linear regression analysis.

Total phenolic content was determined according to the Folin–Ciocalteu method, using gallic acid as a standard (29). The extracted sample was dissolved in methanol/water (50/50 (v/v)). Six hundred microliters of the dissolved sample was mixed with 600 µl of 1N Folin–Ciocalteu reagent. The mixture was allowed to stand for 5 min, and then 1 ml of 20% Na_2CO_3 was added. After a 10-min resting period, the mixture was centrifuged for an additional 10 min (6,000g). Following centrifugation, the absorbance of the supernatant was measured at 730 nm, using a UV-Vis spectrophotometer. The total phenolic content was expressed as the gallic acid equivalent (GAE) in µg/ml of the sample.

The reducing ability was measured following the method described by Singh and Rajini (30). Two hundred microliters of the extract were mixed with 200 µl of 1% (w/v) $K_3Fe(CN)_6$ and 200 µl of 0.2 M phosphate buffer with a pH of 6.6. The mixture was kept at 50°C for 20 min. Two hundred microliters of 10% (w/v) trichloroacetic acid was then added, and the mixture was centrifuged at 3,000 rpm for 10 min. One hundred microliters of the supernatant was transferred to a 96-well plate, with each well containing 100 µl of distilled water and 20 µl of 0.1% (w/v) $FeCl_3$ solution. The absorbance of each well was measured using an ELISA reader at a 700-nm wavelength.

To study the influence of anthocyanins on the UV protection ability, anthocyanin extracts were added to the prepared cosmetic cream at ratios of 0, 5, and 10 ml/100 g of cream, respectively. After each sample was well-mixed, 0.1 g of the cream was dissolved in 2 ml of acidic ethanol (0.1 N $HCl_{(aq)}$ in ethanol, 15:85 (v/v)). The UV absorbance of the solution was measured using a UV spectrophotometer at wavelengths of 300, 325, 350, and

375 nm. The prepared cosmetic cream was also evenly spread on a slide. Two cover slides were placed on the ends of the above slide, and another slide was placed on top of the two cover slides, creating a space with a depth of 0.15 mm, equivalent to the thickness of the spread cream layer. The slides filled with cream were placed under a UV light source (Model# PS-160, Horng & Fung Corporation, Taiwan). A UV light meter (Model# UV-340, Lutron^{R}, Taiwan) was put beneath the slides to measure the UV irradiation through the samples. A blank test was performed to calibrate the effect of the slides on the UV irradiation. All experimental tests were repeated three times.

Data were analyzed using analysis of variance (ANOVA), and the results were compared using the Student *t*-test. A difference was considered to be statistically significant when the *p*-value was less than 0.05 ($p < 0.05$).

RESULTS AND DISCUSSION

The absorbance of ethanol extract diluted 15× in pH 1.0 and pH 4.5 buffers was 0.686 and 0.198, respectively. Based on Equation 1, the total anthocyanin content was calculated to be 122.2 mg/l. The color of the ethanol-extracted anthocyanin solution was reddish-purple with a pH of 4.1. When the pH was raised from 4 to 11 by the gradual addition of 0.1 N $NaOH_{(aq)}$, the solution color changed from pink to green, and finally to yellow. The pH value had a significant effect on the color of the anthocyanin solution. A pH between 4 and 5 was considered ideal, because anthocyanins are more stable under an acidic environment. According to Figure 1, the purple sweet potato extracts demonstrated a good ability to absorb ultraviolet radiation, particularly at wavelengths between 250 and 350 nm. Therefore, the purple sweet potato extracts appear to be an ideal ingredient for improving the cosmetic cream's UV-A and UV-B absorption properties. The results shown in Figure 1 were obtained from solutions diluted 400×. After correcting for the dilution, the UV absorbing ability of the initial extracts was excellent. Although a neutral pH condition resulted in the best UV-absorbing ability, the greenish-yellow color might not appeal to consumers.

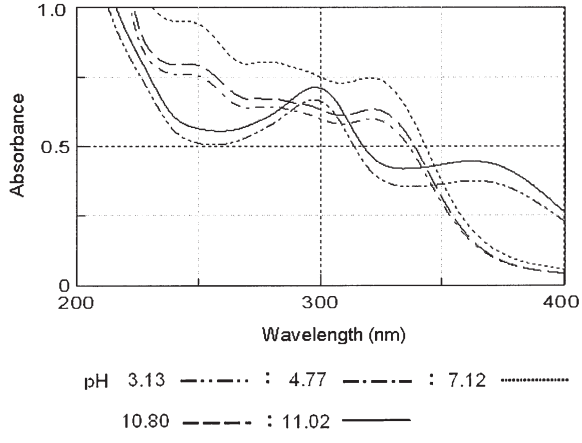

Figure 1. The influence of pH on the absorbance of anthocyanin solution extracted from TNG73 purple sweet potato (with 400× dilution) measured by the spectrophotometric method.

The DPPH radical scavenging activity of the anthocyanin extracts was measured as the decrease in absorbance of DPPH at a wavelength of 517 nm. Figure 2 shows the results that were plotted as a function of anthocyanin extracts. The DPPH radical scavenging activity increased with increasing anthocyanin extract content. The difference was more significant when the anthocyanin extract content was less than 2%. Anthocyanins extracted by using acidic ethanol had a better radical scavenging activity. With a high anthocyanin extract content, the DPPH radical scavenging activity was approximately 78% for acidic ethanol extracts. The DPPH radical scavenging activity of acidic ethanol and acidic water extracts was significantly different ($p < 0.05$) at 0.25%, 1.25%, 2.5%, and 5% anthocyanin extract content. The anti-oxidative activity can be represented by the EC_{50} value, and the results are shown in Table I. The EC_{50} values of acidic ethanol and water extracts were 1.63 ± 0.14% and 3.32 ± 0.22%, respectively. The results revealed that the acidic ethanol extract had better radical scavenging activity than the acidic water extract.

The total phenolic content of the acidic ethanol and acidic water extracts of TNG73 purple sweet potato was expressed as μg GAE/ml sample, and the results are shown in Table II. When 10% extracts were used, the phenolic content analysis results were 97.26 ± 9.07μg/ml and 80.95 ± 8.86 μg/ml for acidic ethanol- and water-extracted samples, respectively. In addition, the phenolic content of the acidic ethanol extract was always higher than that of the acidic water extract.

The reducing ability measured as the change in absorbance with a 700-nm wavelength correlated to the anti-oxidative ability of the anthocyanins. The higher reducing ability

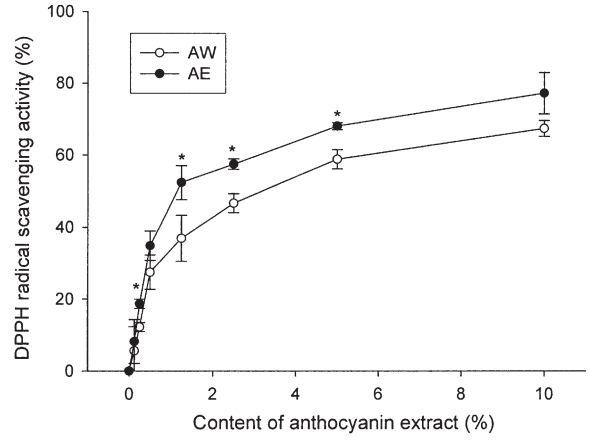

Figure 2. 1,1-diphenyl- 2-picrylhydrazyl (DPPH) radical scavenging activity of acidic ethanol (AE)- and acidic water (AW)-extracted anthocyanin solutions. Each value is the mean ± SD (n=3) of the measurements performed. *Indicates a significant difference ($p<0.05$) between the two groups.

Table I
The EC_{50} Values of Acidic Ethanol (AE) and Acidic Water (AW) Extracts of Purple Sweet Potato

	EC_{50} value (%)
AE extract	1.63 ± 0.14
AW extract	3.32 ± 0.22
Ascorbic acid[a]	82.5 ± 3.3

[a]The unit of EC_{50} of ascorbic acid is μg/ml.

Table II
Total Phenolic Content of Acidic Ethanol (AE) and Acidic Water (AW) Extracts of Purple Sweet Potato

	Total phenolic content (μg gallic acid equivalent/ml sample)			
Extract added (%)	1.25%	2.5%	5%	10%
AE extract	14.08 ± 1.61*	27.38 ± 1.83*	56.26 ± 7.34	97.26 ± 9.07
AW extract	8.96 ± 1.25	18.60 ± 1.62	47.19 ± 5.16	80.95 ± 8.86

Each value is mean ± SD (n=3).
*Indicates a significant difference ($p < 0.05$) between the two groups.

represented the stronger anti-oxidative ability. Figure 3 shows the effect of anthocyanin extracts on the reducing ability of anthocyanins that were extracted using acidic ethanol and acidic water. The reducing ability of acidic ethanol and water extracts was significantly different ($p < 0.05$) at 1.25%, 5%, and 10% of anthocyanin extract content. The relationship between the reducing ability and anthocyanin content was almost linear. Thus, using more anthocyanin extracts resulted in a better reducing ability. Meanwhile, the acidic ethanol-extracted anthocyanins showed a higher reducing ability compared to acidic water-extracted anthocyanins.

According to the above results, using more anthocyanin extracts resulted in better DPPH radical scavenging activity, higher total phenolic content, and higher reducing ability. The acidic ethanol-extracted anthocyanins were good anti-oxidative ingredients for the cosmetic cream. Adding anthocyanin extracts to the cosmetic cream not only provides ultra-UV protective ability, but also helps skin against free radicals.

Figure 4 shows the UV absorption ability of a cosmetic cream at different wavelengths measured by the spectrophotometric method. The addition of anthocyanin extract dramatically improves the UV-absorbing ability of the cosmetic cream for both UV-A (350 and 375 nm) and UV-B (300 and 325 nm), with the improvement being more significant for the UV-B. The cosmetic cream with the anthocyanin extract was more effective for UV-B absorbance than for UV-A. The results revealed that anthocyanin extracts are ideal

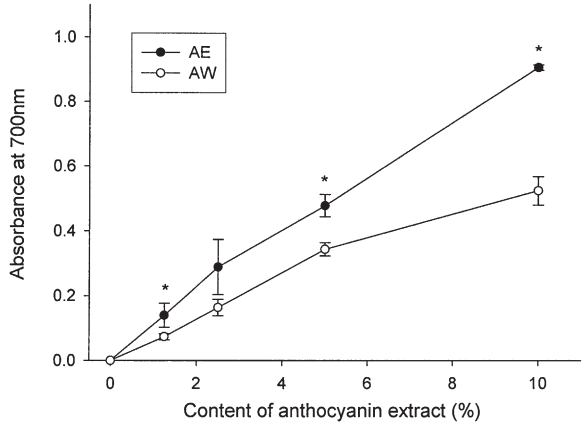

Figure 3. Reducing ability of acidic ethanol (AE)- and acidic water (AW)-extracted anthocyanin solutions. Each value is the mean ± SD (n=3) of the experiments performed. *Indicates a significant difference ($p<0.05$) between the two groups.

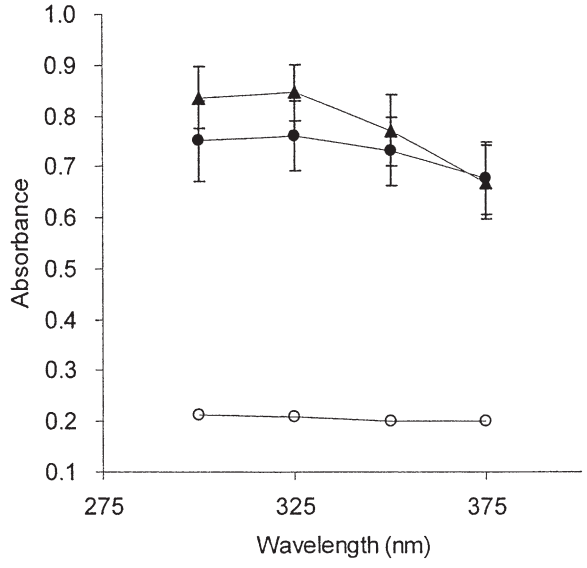

Figure 4. The UV absorbance of a cosmetic cream with the UV-A and UV-B regions measured by the spectrophotometric method (○: no anthocyanin extract added; ●: 0.61 mg/100 g anthocyanin extract added; ▲: 1.22 mg/100 g total anthocyanins added).

ingredients for absorbing UV radiation. Without the addition of anthocyanin extracts, the UV-A and UV-B absorption ability of the cosmetic cream was low.

Figure 5 shows the fraction of incident UV radiation that is absorbed by the cosmetic cream. Without the addition of anthocyanin extract, the cosmetic cream absorbed approximately 30% of the UV radiation. When 5 ml of the anthocyanin extract (0.61 mg of

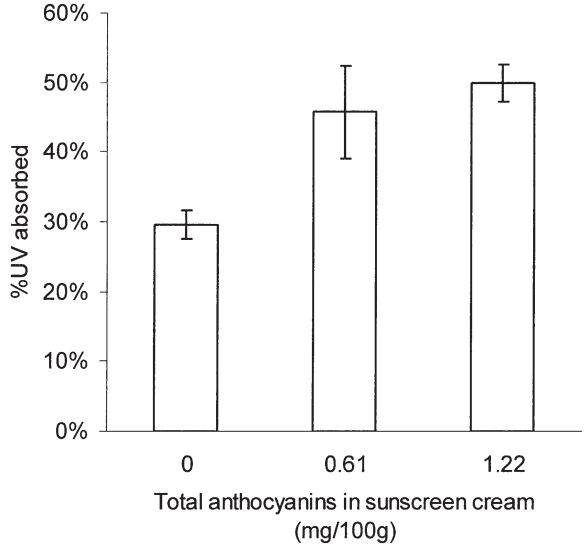

Figure 5. The influence of anthocyanin extract on the percentage UV radiation absorbed.

total anthocyanins) was added to 100 g of the cosmetic cream, the UV absorption ability of the cosmetic cream was significantly improved, reaching 46%. When 10 ml of the anthocyanin extract (1.22 mg of total anthocyanins) was added, 50% of the UV radiation was absorbed. Based on Figure 5, anthocyanin extract contributed to the absorption of UV radiation. However, the relationship between UV absorption and anthocyanin extract content was not linear. The improvement in UV absorption ability became less significant as more anthocyanin extract was added. The optimal anthocyanin extract content is dependent on practical application and requires further study. Generally speaking, a small amount of anthocyanin extract was adequate for significant enhancement of UV absorbing ability.

The recipe provided in this study was designed to show the effect of adding purple sweet potato extract to a cosmetic cream. The UV-absorbing ability of anthocyanin extracts was measured by using a UV spectrophotometer without running expensive human tests. Although the UV spectrophotometric methodology is a convenient tool for preliminary evaluation, human tests are still required to confirm the influence of anthocyanins on humans. The next step of this study is to get governmental permission for running SPF analysis on humans.

CONCLUSIONS

TNG73 purple sweet potato extracts were an excellent anthocyanin source for preparing UV-protective cosmetic cream. The acidic ethanol-extracted anthocyanins had better radical scavenging ability, higher total phenolic content, and stronger reducing ability than anthocyanins extracted using acidic water. Based on these results, we recommended the use of acidic ethanol-extracted anthocyanins as additives in UV-protective cosmetic cream. The UV absorption ability of the cosmetic cream was successfully improved by the addition of anthocyanin extract. The difference in UV absorption ability arising from the addition of anthocyanin extract was more significant for UV-B rays. However, the optimal anthocyanin extract content remains dependent on practical application, and requires further study.

ACKNOWLEDGMENTS

This research was funded by Chang Gung Institution of Technology, grant no. EZRPF380141.

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