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Color profiles and stability of acylated and nonacylated anthocyanins as novel pigment sources in a lipstick model: A viable alternative to synthetic colorants

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Synopsis

Cosmetics, such as lipstick, can affect an individual's perception of attractiveness and morale. Consumer concern with the safety of synthetic colorants has made the need for alternative natural color sources increasingly urgent. Our goal was to evaluate the feasibility of anthocyanin (ACN) extracts as colorants in lipstick formulations. Lipstick formulations were colored with ACN-rich materials. Accelerated environmental testing typical of the cosmetic industry were used: incubation at 20°, 37°, and 45°C for 12 weeks and temperature abuse cycles between 20°/37°C or −20°/20°C. Color (CIELab) and total monomeric ACN (pH-differential) changes were monitored to determine shelf stability of the product. All formulations exhibited acceptable color for lipsticks. Shelf stability was determined to exceed 2 year based on the accelerated testing conditions. Formulations containing cyanidin as their main ACN were the most stable (elderberry, purple corn, and purple sweet potato). ACNs could be used as suitable alternatives to synthetic colorants in lipid-based topical formulations.

INTRODUCTION

Color is a crucial element for the cosmetic industry because it has a direct and immediate effect on consumer self-perception of attractiveness (1). The strongest evidence for the psychological influence of colored cosmetics, such as lipstick, is the phenomenon of increased spending by women on attractiveness-enhancing products during times of economic downturn, nicknamed the "lipstick effect" (2). Lipstick has been associated with boosts in morale, as well as increased attractiveness to potential mates, since the Great Depression when lipstick sales skyrocketed unexpectedly (2).

Lipsticks are typically colored with synthetic pigments such as D&C Reds #6 (CI 15850), #7 (CI 15850), #28 (CI 45410); FD&C Yellow #5 (CI 19140), and FD&C Blue #1 (CI 42090); however, consumer concerns with the safety of synthetic colorants have been growing in recent years. Eosin- and fluorescein-based colorants used in cosmetics [D&C Red #21 (CI 45380), D&C Red #22 (CI 45380), and D&C Red #27 (CI 45410)] are often

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associated with photosensitization-induced cheilitis or inflammation of the mouth (3). Azo dyes are frequently associated with contact dermatitis, especially with repeat exposure (4). Azo dyes, such as D&C Red #6, D&C Red #7, and FD&C Yellow #5, are some of the most commonly used organic pigments in lipstick formulations (5). Carmine (CI 75470), a natural source of color in cosmetics, has also been shown to produce contact dermatitis on the lips and skin of sensitive populations (6,7).

The combination of concerns with synthetic colorants and an increase in consumer demands for more "natural" ingredients in cosmetics makes finding plant-derived alternatives a necessity (8).

One such alternative color source is a group of water-soluble flavonoids known as anthocyanins (ACN) (9). ACNs are responsible for many of the red, purple, and blue shades found in fruits and vegetables. Although ubiquitous in nature, there are six common aglycones, namely, cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin, with various types of glycosylation and acylation. They have been frequently used in the food industry as a natural colorant source (10). Their use as alternatives to synthetic colorants, such as FD&C Red #40 (CI 16035) (11), FD&C Red #3 (CI 45430), and FD&C Blue #2 (CI 73015) (12), has also been proposed. Moreover, the interest in their use as colorants has also been fueled by a desire to find uses for waste by-products of the agriculture industry, such as grape skins from the wine industry (13,14).

The stability and color of ACNs is influenced by many factors including pH, temperature, light exposure, and interactions with other compounds (15). Substitutions on the B-ring of ACNs and the presence of additional hydroxyl or methoxyl groups influence their stability (16). However, ACN sources with additional glycosylations and acylations have been shown to exhibit high resistance to these degradation factors (11).

ACNs have also gained increased interest because of their potential health benefits, such as their potent antioxidant properties (11). Therefore, their use in cosmetics may replace ingredients of concern to consumers with potentially health-promoting bioactive pigments. To date, most of the research surrounding the use of ACNs has been focused on aqueous food systems (17). The potential of certain ACN sources to match the colors typically used in lipstick formulations makes them an attractive alternative to synthetic colorants.

The aim of this study was to investigate the potential use of ACN extracts as natural colorants in lipstick formulations by evaluating their ability to produce shades of lipstick of commercial relevance, as well as their color stability during accelerated environmental testing as viable alternatives to synthetic lipstick colorants. Nonacylated cyanidin–based and acylated cyanidin–based sources, such as elderberry (*Sambucus nigra* L.), purple carrot (*Daucus dacota* L), purple corn (*Zea mays* L.), purple sweet potato (*Ipomoea batatas* L.), and red cabbage (*Brassica oleracea* L.), were chosen because of their vast abundance in nature and high stability (18). Hibiscus (*Hibiscus sabdariffa* L.), a source of nonacylated delphinidin, was chosen because of its reported high antioxidant activity (19). Red radish (*Raphanus sativus* L.), a source of acylated pelargonidin, was chosen because of its reported stability and potential as an alternative to synthetic red colorants (20). Strawberry (*Fragaria x ananassa*), a source of nonacylated pelargonidin, was used for a comparison of acylation effects in pelargonidin (21). Red grape (*Vitis vinifera* L.), which contains all six aglycones, was also investigated to better understand the effect of chemical structure on color stability.

MATERIALS AND METHODS

MATERIALS

Elderberry, hibiscus, purple carrot, purple sweet potato, red cabbage, and red radish dried extracts were provided by DD Williamson & Co., Inc. (Louisville, KY), the strawberry dried extract was provided by FutureCeuticals Inc. (Momence, IL), and the purple corn and red grape skin dried extracts were provided by Artemis International (Fort Wayne, IN). The base of the lipstick formulations and the colorants, D&C Red #6 and #7 (CI 15850), Mica Red, and Carmine (CI 75470), were purchased from MakingCosmetics, Inc. (Snoqualmie, WA). Five commercial brands of lipstick containing synthetic colorants and one brand containing natural colorants were purchased from a local department store (Columbus, OH). Black lip balm containers were purchased from a local company, Bulk Apothecary (Streetsboro, OH). Glass slides were purchased from Fisher Scientific Inc. (Fair Lawn, NJ). Reagents used were acetone, ethanol, and methanol and were purchased from Fisher Scientific Inc.

SPECTROPHOTOMETRIC ANALYSIS OF THE TOTAL MONOMERIC CONTENT

The total monomeric ACN content for the extracts was measured in 1-cm cuvettes using a spectrophotometer (Shimadzu UV-2450 Spectrophometer, Kyoto, Japan) by the pH-differential method as described by Giusti and Wrolstad (22). The absorbance at pH 1.0 was determined for the extracts using a potassium chloride buffer with HCl after a 15-min equilibration time. The absorbance at pH 4.5 was determined for the extracts using a sodium acetate buffer with HCl after a 15-min equilibration time. The ACN content, expressed as cyanidin-3-glucoside, was determined using the following equation:

$$\text{Total monomeric anthocyanin content}\left(\frac{\text{mg}}{\text{l}}\right)=\frac{\text{Abs}_{\text{pH 1.0}}-\text{Abs}_{\text{pH 4.5}}\times\text{DF}\times 1{,}000}{\varepsilon\times d} \quad (1)$$

Where MW = molecular weight of the major ACN present, DF = dilution factor, ε = molar absorptivity of the major ACN present, and d = path length (1 cm). Results were then used to determine the ACN content in the dried extract powder based on initial weights and were recorded as mg/g.

LIPSTICK FORMULATIONS

Formulations were based on the recommendations in the Society of Cosmetic Chemists Monograph Number 8: Lipstick Technology (5). All dried extracts were incorporated as 8% of the final weight (w/w) of each lipstick formulation based on the preliminary data. The dried extracts were initially weighed out and subjected to a grinding process with a mortar and pestle before being added to the lipstick manufacturing. The formulations underwent a wet grinding process in which castor oil was used in a 1:3 ratio (pigment:oil), and silica was included at 1% of the final weight (w/w), to increase uniformity in the final products. Initially, the lipstick base was weighed and placed i n a water bath at 70°C with gentle stirring until completely melted. The preground dried extracts were then poured directly into the hot lipstick base and gentle stirred until a uniform color was achieved. The lipstick formulas were then poured directly into the lip balm containers and allowed to cool at 4°C until completely solid.

ACCELERATED ENVIRONMENTAL TESTING

The parameters used for the shelf-stability testing were based on the guidelines set by The European Cosmetic, Toiletry and Perfumery Association (23), and Cannel (24). The six formulations tested for shelf stability were formulas using elderberry, purple corn, red carrot, grape skin, purple sweet potato, and red radish as their pigment sources, respectively. Once the formulas were allowed to cool, 0.5 g of each was secured between two glass slides for use in the stability testing. The formulas were stored in the dark at each temperature in triplicates. The temperatures tested were 20°, 37°, and 45°C. A baseline color measurement was taken on day 0. Color measurements were taken then on day 1 to account for any changes that may have taken place in the initial 24 h period. Color measurements were then taken on a weekly basis until the conclusion of the testing for each temperature at 12 weeks.

In addition, the formulas were subjected to two different temperature cycles. The first cycle was 20°–37°C. One cycle included being held at 20°C for 24 h and then subsequently placed in 37°C for 24 h. The second cycle aimed to test the formulation stability to freeze/thaw abuse. One cycle included being held at −20°C for 24 h and then subsequently placed in 20°C for 24 h. Color measurements were taken after the completion of each 48-h cycle. The cycles were repeated six times for all samples. The six formulations tested for shelf stability were decided on the preliminary data at 20°C. After the incubation period at each temperature, the samples were collected and placed at −20°C until further analysis.

COLORIMETRIC ANALYSIS

A colorimetric analysis was performed to determine objectively the color characteristics of the initial color of the formulations prepared in the lab and to compare them with the color of commercial products purchased. Color analyses were also used to monitor color changes during different conditions of accelerated environmental testing. Formula color was measured using a reflectance specular included mode, with a D65 light source and 10° observer angle, on a Color Quest XE (Hunter Associates, Inc., Keswick, VA). Equal amounts of sample (0.5 g) were presented to the colorimeter encased between two glass slides, to ensure uniformity between the samples. All readings were replicated ($n = 3$) and reported in the Hunter CIELab system using Easy Match Software Ver3.62 (Hunter Associates, Inc.).

Color measurements were then averaged after each color reading. Changes in L^*, a^*, b^*, c^*, h, and ΔE^* were recorded against the baseline measurements taken on day 0. L^*, is the measurement of the lightness of a sample, with higher values (0–100 scale) indicating a lighter sample. The a^* scale is the measurement of red versus green, where a positive number indicates a red color. Conversely, b^* is a measurement of blue versus yellow, where a negative number indicates a blue color. c^*, or chroma, is a measurement of intensity or saturation of color. The hue angle, $h°$, is a measurement of where the sample color falls on the color wheel. Delta E, ΔE^*, is a mathematical description of the distance between two samples in the $L^*a^*b^*$ color space. The initial color measurements were used as standards for ΔE^* determination based on the following equation:

$$\Delta E = \sqrt{\left[\left(L_i^* - L_0^*\right)^2 + \left(a_i^* - a_0^*\right)^2 + \left(b_i^* - b_0^*\right)^2\right]} \quad (2)$$

EXTRACTION OF ACNS FROM THE FORMULATIONS

To understand the effects the stability testing had on the ACNs, a method for extracting the pigments out of the formulations was developed. Initially, the samples were collected from the microscope slides and weighed. The weighed samples were then powderized using liquid nitrogen and a tissuemeizer (Fisher Scientific Inc.). Three extraction solvents were used to extract the ACNs from the lipstick base in equal parts: acetone (70%), acidified ethanol (0.01% HCl), and acidified deionized distilled water. The samples were vigorously mixed with the solvents using the tissuemeizer. To cause a phase separation, equal parts (v/v) of chloroform was added to each extraction and mixed. The extracts were then centrifuged at 10,000 rpm for 10 min, or until a complete separation was achieved. The aqueous supernatant was then collected in a-250-ml boiling flask, and the excess solvent was evaporated off using a Büchi rotary evaporator (Brinkmann Instruments, Inc., Westbury, NY). The pigments were redissolved in acidified deionized distilled water and brought to a known volume. The extracts were then stored at −20°C until further analysis was performed. Recovery efficiency was determined using the pH-differential method described previously. The recovered extracts were tested for total monomeric content, the results of which were compared with the initial monomeric content and used to determine recovery rates.

STATISTICAL ANALYSIS

A statistical analysis for the ACN content and changes in color measurements was done using one-way analysis of variance and linear regressions using Minitab Statistical Software Version 16 (State College, PA). ($\alpha = 0.05$) and GraphPad Prism Version 6 (La Jolla, CA).

RESULTS AND DISCUSSION

ACN SOURCES AND THE TOTAL MONOMERIC CONTENT

The sources of ACN were initially chosen based on their aglycone profile and acylation (Table I).

The monomeric ACN content was determined to help better illustrate the stability of the pigments in their respective lipstick formulas (25). The total monomeric ACN content was expressed as milligrams of monomeric ACN per gram of dried extract (Table II). The total monomeric concentration ranged from 32.96 mg ACN/g extract for the elderberry powder to 4.38 mg ACN/g extract for the purple carrot powder.

Table I
Primary Aglycones and Acylation Patterns of the ACN Sources Tested

Source	Primary aglycone	Acylation type
Elderberry	Cyanidin	—
Hibiscus	Delphinidin	—
Purple carrot	Cyanidin	Cinnamic acids
Purple corn	Cyanidin, pelargonidin, petunidin	Malonic acids
Purple sweet potato	Cyanidin, peonidin	Cinnamic acids
Red cabbage	Cyanidin	Cinnamic acids
Red grape skin	Delphinidin, cyanidin, Petunidin, malvidin, peonidin,	—
Red radish	Pelargonidin	Cinnamic acid/malonic acids
Strawberry	Pelargonidin	—

All formulations were standardized to include 0.5 g of the ACN-rich extracts to mimic the typical manufacturing procedure of adding colorants by weight. The total amount of ACN (mg/g) added to the formulas can be seen in Table II.

COLOR COMPARISON OF ACN-FORMULAS WITH REFERENCE SAMPLES

Color measurements and visual inspection of the formulations were used to determine suitability of coloration for use in lipsticks when compared with commercially available brands and colorants used in cosmetics (Table III).

Formulations with hue angles similar to those of the reference samples (all except red cabbage) were subjected to preliminary shelf-stability testing for 6 weeks at room temperature and color changes were monitored weekly. Formulations with a ΔE^*, or total change in color, of ≤ 1 were chosen to continue on with stability testing. Only the hibiscus ($\Delta E^* = 12.8$) and strawberry ($\Delta E^* = 15.1$) formulas showed color changes >1, and the significant changes observed for these were deemed unacceptable for further testing.

All chosen formulations fell within the purple to red spectrum based on their hue angles. Elderberry was the most purple formula (initial hue: 318.64°), and red radish was the reddest formula (initial hue: 14.34°). The remaining formulas were varying shades of pink. The hue angles were compared with those of commercially available lipsticks and

Table II
Total Monomeric ACN Content (mg ACN/g Extract) as Determined by the pH-Differential Method for Dried Extracts of the Pigments Tested, and Total Amount Incorporated into the Lipstick Formulas

Dried extract	Total ACN content of pigments	Total ACN content in formula
Elderberry	32.96	16.48
Hibiscus	14.24	7.12
Purple carrot	4.38	2.19
Purple corn	22.27	11.14
Purple sweet potato	19.20	9.6
Red cabbage	26.31	13.16
Red grape	29.86	14.93
Red radish	24.20	12.10
Strawberry	18.36	9.18

Table III
Color and Hue Angle of Preliminary ACN-Colored Lipsticks Compared with Commercial Lipstick Samples and Samples Colored with Non-ACN Pigments

Reference samples[a]			ACN-colored lipsticks			
	Hue	Color		Hue	Color	ΔE^*
Sample 1	21.4	Orange/Red	Strawberry	18.2	Orange/Red	15.1
Sample 2[a]	20.1	Orange/Red	Red radish	14.9	Red	0.7
Sample 3	15.8	Red	Purple sweet potato	1.6	Pink/Red	0.2
Sample 4	15.4	Red	Red grape	1.4	Pink/Red	0.3
Sample 5	14.5	Dark Red	Purple carrot	354.4	Pink	0.4
Sample 6[†]	14.4	Dark Red	Purple corn	350.5	Purple/Pink	0.6
Sample 7	8.0	Berry Red	Hibiscus	349.1	Purple/Pink	12.8
Sample 8	6.8	Pink/Red	Elderberry	318.3	Purple	0.4
Sample 9[‡]	352.0	Pink/Purple	Red cabbage	298.3	Purple/Blue	—

ΔE^* represents the color change over 6-weeks storage at room temperature as compared to their original color for ACN-lipsticks as part of a preliminary selection process.
[a]Reference samples were commercial brands or lipstick formulations prepared with nonanthocyanin pigments formulated with D&C Red #7.
[b]Reference samples were commercial brands or lipstick formulations prepared with nonanthocyanin pigments formulated with Mica Red.[c]Reference samples were commercial brands or lipstick formulations prepared with nonanthocyanin pigments formulated with Carmine.

lipstick formulations using traditional colorants: D&C Red #7 lakes, mica red, and carmine at identical concentrations.

The commercial brands chosen were shades of orange/red (Sample 1 and 2), red (Sample 3 and 4), dark red (Samples 5 and 6), and varying shades of pink-red to purple-red (Brand 7–9). The hue angle for the red radish formulation was the most similar to the commercial brands, especially for Samples 4–6.

The hue angle of red radish was closest to those observed with the D&C #7 sample (Sample 2, hue angle: 20.05°) and nearly identical to the mica red sample (Sample 6, hue angle: 14.43°). The hue angles for purple carrot (initial hue: 354.44°) and purple corn (initial hue: 350.45°) were very similar to those observed with the carmine lipstick (Sample 9, hue angle: 352.03°). The hue angles for the red grape (1.42°) and purple sweet potato (1.61°) fell slightly above those seen with the carmine lipstick; however, all three were a reddish-pink hue similar to Samples 7–9. The elderberry formulation was more purple than the commercial samples but was still deemed visually acceptable for use in a lip product.

COLOR CHANGES DURING AN ACCELERATED ENVIRONMENTAL TESTING

Accelerated environmental testing conditions were implemented to help predict the shelf stability of the ACN-based lipstick formulations. Color measurements, as well as visual inspections, were used to assess the samples at each condition. An accelerated environmental testing is commonly used in the cosmetic industry to predict shelf life of new products (24). Although each company will have their own set of testing parameters, it is commonly assumed that a product showing stability after 10 weeks at 45°C will have an estimated shelf life of 2 year at room temperature (26). Moreover, it is generally recommended that lipsticks should be discarded after 2 year and “natural” lip products be discarded within 12 mo of opening, known as the period after opening (27). These recommendations were used as a guideline for interpreting the results of the stability testing.

Initial and final measurements for L^*, a^*, c^*, $h°$, and dE^* all formulas can be seen in Table IV. Although individual variations occurred within groups and between formulations, some generalizations can be observed. Overall, increased stability was shown with formulations containing cyanidin (elderberry) and acylated cyanidin (purple corn and purple sweet potato).

Changes in lightness (L^*) were minute for all formulas across all treatments (≤2.5). All samples were slightly darker at the conclusion of the 12 weeks at 45°C. Changes in redness (a^*) of the samples were not statistically significant (p value ≤ 0.01) for all treatments, with the exception of the red radish formula at 37° and 45°C. The elderberry and purple corn formulations showed the smallest changes in a^* across treatments, and the red grape and red radish showed the greatest changes. Changes in chroma (c^*), followed a similar pattern with changes in a^*, with the smallest changes observed with the elderberry and purple corn lipsticks. Interestingly, both elderberry and purple corn formulas showed increased color intensity at 20°C after 12 weeks.

Table IV
Initial and Final CIELab Color of ACN-Lipstick Formulations at Accelerated Environmental Testing Conditions

Elderberry						Purple sweet potato					
Temperature	L^*	a^*	c^*	$h°$	ΔE^*	Temperature	L^*	a^*	c^*	$h°$	ΔE^*
Initial	29.09	1.80	2.41	318.64	0.00	Initial	29.98	6.43	6.45	361.61	0.00
−20°/20°C	29.33	2.39	2.92	325.40	0.73	−20°/20°C	31.98	6.14	6.16	355.90	0.66
20°/37°C	29.18	1.94	2.47	321.98	0.26	20°/37°C	30.22	5.53	5.54	359.36	1.09
20°C	28.78	3.07	3.31	338.45	1.78	20°C	31.54	5.85	5.89	354.17	1.99
37°C	27.40	2.99	3.19	339.29	1.97	37°C	30.18	4.28	4.40	346.65	2.80
45°C	27.99	1.94	2.29	328.24	1.27	45°C	28.82	4.52	4.57	351.43	2.36
Purple carrot						Red grape					
Temperature	L^*	a^*	c^*	$h°$	ΔE^*	Temperature	L^*	a^*	c^*	$h°$	ΔE^*
Initial	33.63	7.97	8.01	354.44	0.00	Initial	35.30	9.66	9.66	1.42	0.00
−20°/20°C	33.95	7.55	7.61	353.27	0.93	−20°/20°C	34.85	9.47	9.47	0.59	0.41
20°/37°C	33.76	7.22	7.28	352.53	0.44	20°/37°C	34.88	9.16	9.16	0.81	0.33
20°C	32.85	9.25	9.27	356.63	1.28	20°C	36.88	9.25	9.27	3.33	0.89
37°C	33.93	8.87	8.92	355.25	1.07	37°C	33.00	8.94	8.96	2.79	1.65
45°C	32.09	6.29	6.45	347.25	3.21	45°C	32.82	6.95	6.96	0.19	3.95
Purple corn						Red radish					
Temperature	L^*	a^*	c^*	$h°$	ΔE^*	Temperature	L^*	a^*	c^*	$h°$	ΔE^*
Initial	28.65	3.72	3.77	350.45	0.00	Initial	29.37	13.75	14.20	14.34	0.00
−20°/20°C	29.54	4.08	4.13	350.72	0.81	−20°/20°C	30.54	13.07	13.45	13.51	0.68
20°/37°C	28.83	3.67	3.73	350.34	0.38	20°/37°C	29.97	12.15	12.35	10.26	1.76
20°C	27.42	4.81	4.83	355.92	1.65	20°C	29.47	11.62	11.82	10.42	1.80
37°C	26.75	3.77	3.83	354.52	1.68	37°C	28.48	9.57	9.66	7.68	3.83
45°C	27.09	3.90	3.94	352.35	1.18	45°C	28.75	9.22	9.30	7.36	4.22

Measurements presented for cycling conditions (−20°/20°C and 20°/37°C) are after six 48-h cycles. Measurements shown for constant accelerated conditions (20°, 37°, and 45°C) are after 12 weeks incubation. All results shown are means (n = 9).

Changes in hue angle varied between formulations, with the greatest changes observed with the elderberry and purple sweet potato formulas. The elderberry lipstick hue angles increased for all conditions, toward a more red hue angle. Conversely, the purple sweet potato lipstick hue angles decreased for all conditions, toward a more purple hue angle. The red radish lipstick hue angles also decreased for all conditions, toward a more red hue angle. Very little changes were observed in hue angle for purple carrot, red grape, and purple corn formulations.

Delta *E* (ΔE^*), or the total color difference, can be used to quantify changes in color (28). Just noticeable differences in color can be detected at a Delta *E* around 2.36; however, unexperienced observers notice differences in color when the Delta *E* is between 3.5 and 5 (28). Ten weeks at 45°C is considered a predictor for shelf stability of 2 year (24); therefore, some color changes were expected by week 12. Red radish formulations showed the greatest ΔE^* after 12 weeks at 45°C (ΔE^*: 4.22), and elderberry and purple corn formulas showed the smallest ΔE^* after 12 weeks at 45°C (ΔE^*: 1.27 and 1.18, respectively). Based on the detection limits of color differences, it was determined that the color changes recorded for the formulas at all conditions were within acceptable limits; therefore, shelf stabilities of at least 2 year were predicted. In addition, the samples were stored in such a way to mimick an opened container, which increases the assurance of their stability.

CHANGES IN TOTAL MONOMERIC CONTENT

The recovered percentage of monomeric ACN content for the formulations were determined based on the initial monomeric content by weight, after the accelerated environmental testing. In general, the highest recovery was seen for the freeze/thaw cycles for all formulations, with a recovery of 98.91% (±0.22) for purple corn to 91.72% (±0.18) for red grape. The recovery at 45°C was the lowest for all formulations, with a recovery of 93.81% (±0.24) for purple corn to 59.11% (±1.40) for red grape. The recovery rates for the formulations followed the same pattern for all accelerated environmental testing. In order of highest recovery the formulations were purple corn, purple sweet potato, elderberry, red radish, purple carrot, and finally red grape (See Figure 1).

Overall, the percentage of recovered ACNs from the formulations followed a similar pattern shown with the color measurements reported previously. These results were expected because of the association of changes in color with degradation, or changes in chemical structure, of ACNs (11). It is not surprising then that the formulations that showed the smallest changes in color, such as the purple corn and elderberry, should have the highest recovery of monomeric ACN content, depending on the storage conditions. It is interesting to note that although the red radish had the greatest change in color, its recovery rate was higher than that of the purple carrot or red grape for all testing conditions. This may be due to the low initial monomeric content, as seen with the purple carrot. Another possibility is that some irreversible binding occurred between the ACN and lipstick matrix, as may be the case for the red grape, which had the lowest recovery for all testing conditions.

CONCLUSIONS

ACNs incorporated into the matrix of lipstick formulations proved to be stable, even under the accelerated environmental testing used to predict a shelf life of at least 2 year.

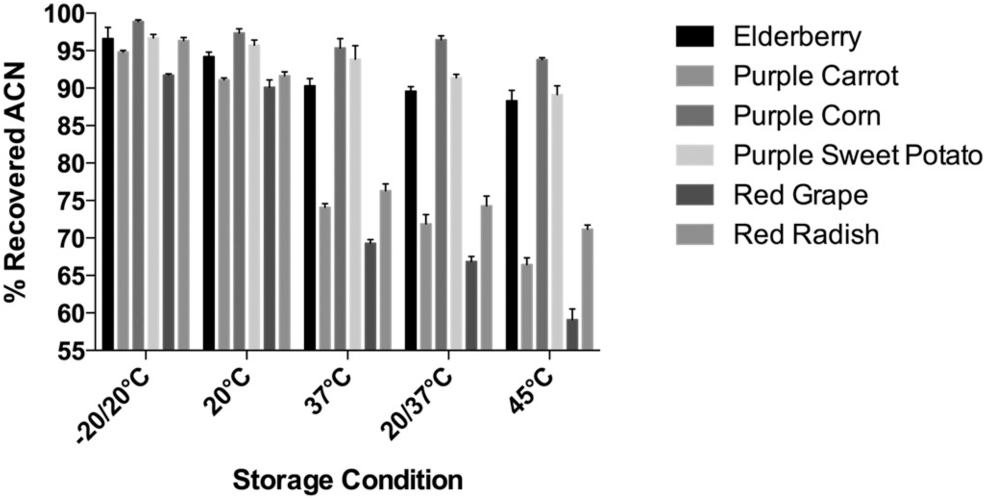

Figure 1. Percentage of recovered ACNs from formulations after 12 weeks of accelerated environmental testing. Percentages based on the initial total monomeric content by weight determined on day 1 ± S.D. ($n = 3$) by the pH-differential method.

There were, however, variations in stability noted with the different sources based on the colorimetric analysis and percentage of recovered ACNs. Sources containing both non-acylated cyanidin–based and acylated cyanidin–based ACNs, such as the elderberry, purple corn, and purple sweet potato, were shown to have the greatest stability when compared with the purple carrot, red grape, and red radish formulas. The red radish formulations showed the closest color characteristics to red colorants used in cosmetics (D&C Red 7; and Mica Red); although it showed the largest change in color, these changes were barely noticeable to the human eye. Therefore, it showed great potential as a natural colorant for the industry.

Overall, our results show the potential for ACNs to be used as stable alternatives to synthetic colorants in the cosmetic industry. ACNs are of particular interest as cosmetic colorants because of their synergistic characteristics of being from natural sources and their potential to act as bioactive ingredients. Investigations are currently being carried out to better understand the role they may play as health-promoting ingredients in cosmetics.

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Graphical Abstract

HIGHLIGHTS

- Sources representing the six major ACN aglycones were evaluated for their use in cosmetic formulations.
- Color profiles of formulas were compared with those of commercially available products and their equivalents.
- Formulas showed good stability to accelerated environmental testing conditions at elevated temperatures.
- Our results suggest a novel application of ACNs as potential alternatives to synthetic colorants in cosmetics.