PLANT SCIENCE TODAY
Vol 12(sp3): 01–04
https://doi.org/10.14719/pst.8249

eISSN 2348-1900

RESEARCH ARTICLE

Efficacy of ascorbic acid and sugar as photo protectants of anthocyanin in grape waste products

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Received: 14 March 2025; Accepted: 16 May 2025; Available online: Version 1.0: 03 October 2025

Cite this article: Gurumeenakshi G, Shanthi K. Efficacy of ascorbic acid and sugar as photo protectants of anthocyanin in grape waste products. Plant Science Today. 2025;12(sp3):01–04. https:/doi.org/10.14719/pst.8249

Abstract

Anthocyanin (ACN), a potent antioxidant is gaining momentum in the recent days, in terms of nutraceutical foods for therapeutic application. One of the major constraints in converting this pigment into a nutraceutical product is by maintaining its stability under light, hence, the food industries generally employ light-protective packaging to inhibit. To achieve, stability of ACN under visible light spectrum, its' imperative to use photo protectants This critical need to elucidate strategies for preserving ACNs against light-induced degradation formed the basis of the current investigation. Therefore, this research was undertaken, to study the influence of the photo protectants, such as ascorbic acid and sugar at varying concentrations in preventing degradation of ACN extracted from grapes processing wastes. The study proved that the addition of ascorbic acid (0.05 %) and sugar (10 %) significantly enhanced ACN stability, retaining 60 % of its content post exposure to light in comparison to the control.

Keywords: anthocyanin; ascorbic acid; photo protectants; stability; sugar; visible light spectrum

Introduction

Nutraceuticals are foods containing bio active compounds derived from plant foods, but they are not consistently used in the mainstream diet. Numerous researchers have demonstrated that the efficacy of such plant derived products in reducing or preventing chronic health diseases is mostly attributed to their non-nutrient secondary metabolites or phytochemicals. One of the notable nutraceuticals from fruits and vegetables that has caught the attention of researchers and food industries are the ACNs. ACNs are of particular interest to the food colorant industry due to their ability to impart vibrant colors. In the recent times, ACNs are also recognized as a potential complementary management tool for the rising chronic health diseases such as cardiovascular problems, neurological disorders, diabetes and cancers. Apart from this, ACNs are also portray as an extensively studied antiaging substance of all times, starting from the "Red wine" paradox to the recent edible cosmetics. These facts lead the researchers and health practitioners to investigate and implore the use of ACN as a health promoting food (1).

Though ACN is an established nutraceutical, it poses certain challenges to convert this embedded plant pigment into a nutraceutical product, retaining its originality. Extensive research constantly highlights the easily degradable nature of ACNs and various factors that contribute to it. ACNs are sensitive to factors like temperature, light, and pH (2, 3). The most challenging aspect of research in ACN is its difficulty in achieving stability under light. Both the extraction of ACN and its application in food products are photo dependent. The current procedure for extraction and food products processing are done in the presence of light. Moreover, developing a methodology to extract ACN in dark would be a complex, costly and non- user-friendly process. Therefore, it is a critical need to achieve the stability of ACN in the presence of light. Visible light still has negative effects on the food component which depends on the lights' intensity and wavelength. Extraction and storage of ACN under visible light along with photoprotectants can be one of the effective strategies to protect it from degradation, which constitutes the objective of the present investigation.

The scope to maintain the stability of ACNs can be enhanced through the process called co-pigmentation and photo protectants. One of the demonstrations of co-pigmentation in the earlier days (4-6) revealed that, acylated ACNs containing two or more aromatic acyl groups may affect the color through a mechanism called intramolecular co-pigmentation. Co-pigmentation also plays a crucial role in wine ageing and maturation (7).

Protective effects of ascorbic acid, on ACNs have been well studied as well. Compound structure of individual ACNs was found to have a marked effect on their reactivity toward ascorbic acid and, consequently, on their resistance toward color fading. In the presence of metal ions, complexes between ACNs and ascorbic acid may be formed, presumably protecting ascorbic acid against oxidation. Protective effects exhibited by ascorbic acid may also be due to its redox

potential, which enables the reduction of ACN *o*-quinones thereby, inhibiting them from further polymerization reactions (8).

Sucrose protected ACNs from degradation during frozen storage and prevented browning and the formation of polymeric pigments. This may probably be attributed to the inhibition of enzymatic reactions or the hindrance of different condensation reactions by sucrose (9). Moreover, the lowering of water activity by sugars may confer protection against ACN degradation. The effect of added sugar on the ACN stability depends on its structure, concentration and type of sugar

Red cabbage is one of the source with high content of ACNs and high potential yield per unit area. ACN extracted from Red cabbage is known to have considerable amounts of mono- or diacylated cyanidin ACNs. The main ACN identified in red cabbage is cyanidin-3-diglucoside-5-glucoside, followed by cyanidin-3-(sinapoyl)(sinapoyl)-diglucoside-5-glucoside and cyanidin-3-(p-coumaroyl)-diglucoside-5-glucoside (10). The type and the degree of acylation of ACNs are the two important factors determining their color characteristics at certain pH values. Owing to its varying ACN compositions, red cabbage ACN extracts can exhibit a wide spectrum of color, ranging from orange through red to purple and blue based on the pH of the surrounding environment. In contrast, ACNs from other plants, like grape skin, black currant and elderberry, exhibits a reasonable degree of coloration only at pH values < 4 (11).

Numerous investigations have been done to study the effect of heat andair in the degradation and stabilization of ACN, but only limited research has been performed to analyse these properties under light. Various researchers have repeatedly attempted to hide ACN from light instead of exposing it to light with photoprotectants. As researchers have emphasised to store ACN containing food products away from light, food industry is in the lookout for packaging material which has become a costly affair. This discourages the food industry to use ACN as a natural colourant and instead use it as a nutraceutical ingredient in functional foods.. Therefore, the current study was attempted to demonstrate that ACN can be protected from light-induced degradation t the use of natural photo protectants.

Materials and Methods

Materials

The Grape pomace used in the study was collected from M/S KRS Organic farms &processing units, Coimbatore. The main chemicals used in this study were all of AR/GR grade.

Methods

Extraction of ACN from grape pomace

Grape pomace was used for ACN extraction method (12). 1000 g grape pomace was crushed into very fine pieces and was blended in a blender after an addition of 1000 mL solvent (1000 mL water and 1 g citric acid). The solution was then kept in the dark for 30 min, followed by filtration and repeated washing of the sediment with 200 mL of the solvent during filtration to ensure maximum extraction of ACN. The obtained concentrate was further filtered through Whatman No.1 filter paper with a vacuum pump and was subsequently concentrated using a rotary evaporator at 45 °C and 175 mbar. Finally, the extract was centrifuged at 6000 rpm for 15 min and a pure sample of ACN extract was obtained. The extract was then stored in a glass bottle covered by aluminium foil and kept at room temperature prior to analysis and was used as the control.

ACN extract with sugar and modified pH

The ACN extract was supplemented with sugar at varying concentrations of 10 and 15 %. The pH of the samples was adjusted to 3 and 5. Subsequently, five sets of samples were obtained as (i) Control (ii) ACN with 10 and 15 % sugar with pH 3, (iii) ACN 10, 15 % sugar with pH 5.

ACN extract with sugar, ascorbic acid and modified pH

To the ACN extract, varying concentrations of sugar and ascorbic acid were added. The pH of the samples was adjusted to 3 and 5. Accordingly, five sets of samples were obtained as (i) Control (ii) ACN with 10 % and 15 % sugar and 0.5 % ascorbic acid at pH 3, (iii) ACN with 10 % and 15 % sugar and 0.5 % ascorbic acid at pH 5.

The entire sample set was exposed to visible light for a period of 8 hrs at a temperature of 32-38 °C. The total ACN content and the colour value was determined after the exposure in all the samples.

Determination of total ACN content (TAC)

The total ACN content of the samples was measured by using pH differential method (13). The ACN extracts were measured at an absorbance of 520 and 700 nm after 15 min of incubation at room temperature (absorbance of sample in pH 1 buffer at 520 nm should be within a range of 0.2-0.8). The TAC is expressed as cyanidin-3-glucoside (C3G) equivalent, which can be calculated by using the following equation (14);

$$\text{TAC (mg/l)} = \frac{A}{\varepsilon l} \times MW \times DF \times 10^3 \qquad \text{(Eq. 5)}$$

where, $A = (A_{520\,nm} - A_{700\,nm})_{pH1.0} - (A_{520\,nm} - A_{700\,nm})_{pH4.5}$; MW is molecular weight (449.2 g/mol) of Cyanindid-3-gluciside; DF is dilution factor; l is path length in cm; ε is molar absorptivity (26,900), and 10^3 is factor for conversion from g to mg.

Determination of color parame*ter*

The color change was determined by using a color meter (Colorflex 45/0, HunterLab). The result was expressed in terms of CIE L*a*b* value, chroma and hue value. In this coordinate system, L* value is a measure of lightness ranging from 0 (black) to 100 (white); a* value ranges from-60 (greenness) to +60 (redness) and b* value ranges from -60 (blueness) to +60 (yellowness). As the values of a* and b* rise, the color becomes more chromatic or saturated. These values approach zero for neutral colors (15). chroma, hue and ΔE is the total color change calculated (16):

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad \text{(Eq. 7)}$$

$$\text{Chroma } (C^*) = \sqrt{(a^{*2} + b^{*2})} \qquad \text{(Eq. 8)}$$

$$\text{Hue } (h^o) = \tan^{-1}(b^*/a^*) \qquad \text{(Eq. 9)}$$

Statistical analysis

One-way analysis of variance (ANOVA) was performed by SPSS 20.0 software (SPSS, Inc., Chicago, IL, USA). Tukey's HSD (honestly significant difference) test was used to compare mean values at the 5 % level of significance ($p < 0.05$).

Results and Discussion

Color parameters

Chroma value (C*)

chroma values of ACN samples, with and without sugar and ascorbic acid are shown in Table 1. These values are also indicators of ACN degradation in the samples. The chroma and hue values were comparatively higher in the treated samples than in the control, both before and after exposure to visible light. From Table 1, it may also be noted that, the samples treated with 15 % sugar with a pH of 5 exhibited the most desirable colour of ACNs in terms of chroma and hue angle value. This indicates that sugar and ascorbic acid harbours photoprotective activity, thereby preventing degradation of ACN under light.

Studies indicated that sugar played a relevant role in protecting ACN in black currant juice extracts (17). At low pH values (2.8), high sugar concentrations (600 g/L) seemed to have a protective effect on the ACNs during heat treatment at 80 °C for 300 min over the course of juice processing, whereas a contrary effect was observed at a higher pH of 4.6. The present study states that sugar is effective even at pH 5, provided the samples are not subjected to thermal processing. The studies revealed that the addition of 10 % sugar to blackberry juice at pH 2 decreased ACN degradation during heat treatment (18).

Studies revealed that, extracted ACN pigments from *Morus nigra* L., *Morus alba* var. *nigra* and *Fragaria* L. when exposed to varying concentrations of sucrose *viz*., 20 %, 40 % and 60 % demonstrated a protective effect on ACNs at a concentration of 20 % sucrose has. However, at higher concentrations, this effect was observed to decrease (19). The present investigation also confirms that sugar has protective effect on ACN, but only under specific pH and medium concentrations.

Similarly, as evident from the Table 2 of ascorbic acid treated samples, the most desirable appeal of colour and the chroma and hue angle values were exhibited by the samples treated with 15 % sugar and 0.5 % ascorbic acid at pH 5. The hue angle is an explanation for fading of colour in the sample and in the present investigation, the fading was predominant in the control samples than in the treated samples. These findings clearly prove that the addition of sugar and ascorbic acid reduces the fading of colour on exposure to visible light. An ascorbic acid concentration of 0.05 % is required to establish the photo protectant activity of ascorbic acid, which is in line with the previous studies demonstrating the effect of light intensity and wavelengths on ascorbic acid concentration and the antioxidant system in tomato fruit grown *in vitro* (20).

The results of the total ACN content are consistent with the chroma and hue values (Table 3). The control samples exhibited the highest ACN degradation than the treated samples. Among the treated group, the sample treated with 15 % sugar and ascorbic had the highest retention of ACN, despite its' exposure to visible light spectrum. These results endorse the efficacy of sugar and ascorbic acid as a photo protective agent to prevent degradation of ACN.

Conclusion

The present study clearly demonstrated the effect of sugar and ascorbic acid as a protecting agent of ACN from degradation under white light. The standardized amount of

Table 1. Change in the chroma and hue angle value of anthocyanin before and after exposure

pH	Treatment	Chroma		Hue angle	
		Before	After	Before	After
3	ACN (control)	$14.34^{b} \pm 0.05$	$8.87^{a} \pm 0.02$	$-30.39^{a} \pm 0.87$	$-42.85^{b} \pm 0.09$
	ACN+Sugar 10%	$14.70^{b} \pm 0.10$	$12.59^{a} \pm 0.23$	$-32.69^{a} \pm 0.34$	$-37.43^{b} \pm 0.29$
	ACN+Sugar15%	$14.74^{b} \pm 0.13$	$13.98^{a} \pm 0.05$	$-27.37^{a} \pm 0.48$	$-36.50^{b} \pm 0.43$
5	ACN (control)	$14.84^{b} \pm 0.03$	$9.27^{a} \pm 0.02$	$-29.26^{a} \pm 0.87$	$-37.45^{b} \pm 0.09$
	ACN+Sugar 10%	$15.23^{b} \pm 0.13$	$14.12^{a} \pm 0.05$	$-22.37^{a} \pm 0.48$	$-33.50^{b} \pm 0.43$
	ACN+Sugar15%	$15.87^{b} \pm 0.13$	$14.93^{a} \pm 0.05$	$-20.12^{a} \pm 0.48$	$-31.20^{b} \pm 0.43$

*a,b,c,.... indicate the significant difference (p<0.05) between before and after exposure within the same pH and treatment

Table 2. Chroma and hue angle value of anthocyanin samples with and without ascorbic acid at different ratios and pH levels, before and after exposure to visible light

pH	Treatment	Chroma		Hue angle	
		Before	After	Before	After
3	ACN (control)	$14.34^{b} \pm 0.05$	$8.87^{a} \pm 0.02$	$-30.39^{a} \pm 0.87$	$-42.85^{b} \pm 0.09$
	ACN+ Sugar 10 % + Ascorbic acid 0.5 %	$16.17^{b} \pm 0.13$	$15.10^{a} \pm 0.05$	$-29.12^{a} \pm 0.48$	$-30.46^{b} \pm 0.43$
	ACN+Sugar15 %+ Ascorbic acid 0.5 %	$16.83^{b} \pm 0.15$	$16.10^{a} \pm 0.06$	$-23.22^{a} \pm 0.42$	$-25.26^{b} \pm 0.23$
5	ACN (control)	$15.24^{b} \pm 0.05$	$9.27^{a} \pm 0.02$	$-29.32^{a} \pm 0.87$	$-42.85^{b} \pm 0.09$
	ACN+ Sugar 10 % + Ascorbic acid 0.5 %	$17.12^{b} \pm 0.14$	$16.58^{a} \pm 0.05$	$-21.12^{a} \pm 0.22$	$-23.06^{b} \pm 0.13$
	ACN+Sugar15 %+ Ascorbic acid 0.5 %	$17.56^{b} \pm 0.15$	$17.12^{a} \pm 0.05$	$-19.12^{a} \pm 0.24$	$-20.06^{b} \pm 0.14$

Table 3. Total ACN content before and after exposure to visible light

pH	Treatment	Chroma	
		Before	After
3	ACN (control)	$24.23^{a} \pm 0.96$	$20.13^{a} \pm 0.36$
	ACN+ Sugar 10 % + Ascorbic acid 0.5 %	$31.08^{b} \pm 0.53$	$26.02^{b} \pm 0.23$
	ACN+Sugar15 %+Ascorbic acid 0.5 %	$33.07^{b} \pm 0.28$	$28.17^{b} \pm 0.29$
5	ACN (control)	$27.83^{a} \pm 0.96$	$22.43^{a} \pm 0.36$
	ACN+ Sugar 10 % + Ascorbic acid 0.5 %	$35.27^{c} \pm 0.23$	$32.27^{c} \pm 0.25$
	ACN+Sugar15 %+Ascorbic acid 0.5 %	$38.61^{b} \pm 0.78$	$36.19^{b} \pm 0.35$

Sugar and ascorbic acid is 15% and 0.5% respectively at a pH of 5. This combination in the ACN extracts from grape pomace exhibited the most desirable colour and minimum degradation when exposed to visible light. The study indicates that the addition of sugar and ascorbic acid can be used as a natural preservative for stabilizing ACN colour and its properties in the development of any kind of food products. This combination is more suitable for development of nutraceutical beverages, functional food salads, soup mixes and various food-based reserves. This will also minimise the reliance in synthetic food colours which are currently the most trusted alternates in the food industry.

Acknowledgements

The authors acknowledge Tamil Nadu Agricultural University for providing the resources and general support for the work.

Authors' contributions

GG conceived the original idea, designed the research, executed the study, interpreted the results, and drafted the manuscript. SK contributed to the interpretation of results and assisted in editing the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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