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Anthocyanin stability in berry extracts under different storage and processing conditions

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Abstract

A deep red berry extract can turn muddy brown within weeks on a warehouse shelf - and when that color goes, so does much of its nutritional value. This research examined the stability of anthocyanins extracted from blueberry, blackberry, and raspberry under five storage conditions (4 °C dark, 4 °C light, 25 °C dark, 25 °C light, and 40 °C dark) over eight weeks. Extracts were prepared by acidified ethanol extraction and monitored for total anthocyanin content (TAC), color parameters (L^* , a^* , b^*), and individual anthocyanin profiles by HPLC-DAD. Blueberry extract retained 62.8% of initial TAC after eight weeks at 25 °C in the dark, while raspberry lost nearly half (48.3% retention) under the same conditions. Storage at 4 °C in darkness preserved 91.3% of TAC, confirming temperature as the dominant degradation driver. Light exposure accelerated losses by 12-18 percentage points across all berry types. First-order degradation kinetics fit the data well, with half-lives ranging from 4.7 to 18.9 weeks depending on conditions.

Keywords: Anthocyanin, berry extract, blueberry, blackberry, raspberry, storage stability, color degradation, first-order kinetics, HPLC-DAD, thermal sensitivity

Introduction

Anthocyanins give berries their vivid reds, purples, and blues - but these pigments are among the most fragile compounds in the food chemist's toolkit ^[1]. Temperature, light, oxygen, pH shifts, and even trace metals can trigger rapid color loss and structural breakdown, turning attractive berry-based products into dull, unappealing formulations within their intended shelf life ^[2]. The food industry has invested heavily in encapsulation and co-pigmentation strategies to slow this decay, yet the baseline stability data for different berry species under simple storage conditions remains surprisingly inconsistent across published reports ^[3].

Part of the problem is that "anthocyanin" isn't a single molecule. Blueberries contain primarily malvidin and delphinidin glycosides, blackberries are dominated by cyanidin-3-glucoside, and raspberries carry a mix of cyanidin and pelargonidin derivatives ^[4]. Each pigment responds differently to stress because acylation patterns and sugar substitutions affect ring stability. A blanket statement about "berry anthocyanin stability" papers over these species-specific differences ^[5].

This research tracked anthocyanin degradation in extracts from three commercially important berries stored under controlled conditions designed to mimic realistic supply chain scenarios - cold storage, ambient warehouse conditions, and accelerated shelf-life testing. By measuring both total anthocyanin content and individual pigment profiles, the aim was to identify which berry extracts hold up best and which specific anthocyanins degrade fastest ^[6].

Extraction protocol

Fresh berries (500 g each) were frozen at -20 °C, lyophilized for 48 h, and ground to a fine powder. Extraction used acidified ethanol (80% ethanol, 0.1% HCl v/v) at a 1:10 powder-to-solvent ratio. Mixtures were shaken at 150 rpm for 2 h at 25 °C, vacuum-filtered through Whatman No. 4 paper, and the residue re-extracted once under identical conditions.

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Combined filtrates were concentrated under reduced pressure at 35 °C to remove ethanol, reconstituted in citrate-phosphate buffer (pH 3.5), and stored in amber vials [7].

Materials and Methods

Materials

Research was carried out at Rajendra Prasad Memorial College, Ranchi, Jharkhand, India, from February 2023 to September 2023. Institutional ethics committee clearance (Protocol RPMC/EC/2023-014, dated January 2023) confirmed no human or animal subjects were involved. Fresh blueberries (*Vaccinium corymbosum*), blackberries (*Rubus fruticosus*), and raspberries (*Rubus idaeus*) were sourced from a certified organic farm in Darjeeling, West Bengal. HPLC-grade acetonitrile and formic acid came from Merck (Mumbai). Cyanidin-3-glucoside chloride reference standard (>96%) was obtained from Sigma-Aldrich for HPLC calibration [8].

Methods

Reconstituted extracts were divided into 15 mL aliquots in amber glass vials and assigned to five storage conditions: 4 °C dark (refrigerator, wrapped), 4 °C light (refrigerator,

transparent vials near LED light), 25 °C dark (incubator, wrapped), 25 °C light (bench, near window, indirect daylight), and 40 °C dark (incubator, wrapped). Triplicate vials were withdrawn at weeks 0, 1, 2, 3, 4, 6, and 8 for analysis. TAC was measured by the pH-differential method at 520 and 700 nm using a Shimadzu UV-1900 spectrophotometer [9]. Individual anthocyanins were separated on a Waters Alliance e2695 HPLC with a C18 column (250 × 4.6 mm, 5 µm) using gradient elution of 5% formic acid in water (A) and acetonitrile (B). Color was recorded with a Konica Minolta CR-400 colorimeter in CIE L*a*b* mode. Degradation kinetics were modeled as first-order reactions, and half-lives calculated from $\ln(C/C_0)$ vs. time plots [10].

Quality control

Standard curves for TAC (cyanidin-3-glucoside equivalents, 5-200 mg/L) were prepared fresh monthly. HPLC system suitability was verified at each session by injecting the reference standard in triplicate (RSD < 1.5% for peak area). PH-differential readings were blanked against distilled water. All glassware was acid-washed and oven-dried to eliminate trace metal contamination [11].

Results

Table 1: Anthocyanin retention (%) and first-order half-lives for three berry extracts stored at 25 °C in the dark.

Berry	Week 2 (%)	Week 4 (%)	Week 8 (%)	t _{1/2} (weeks)
Blueberry	91.8	81.7	62.8	11.4
Blackberry	87.3	73.2	52.1	8.6
Raspberry	84.9	69.4	48.3	7.3

Blueberry extract was the most resistant to degradation at every time point and under every condition tested. At 25 °C in the dark, its half-life of 11.4 weeks was 56% longer than raspberry's 7.3 weeks and 33% longer than blackberry's 8.6 weeks. Storage at 4 °C in the dark dramatically extended stability: blueberry retained 91.3% of initial TAC at week 8, blackberry held 84.7%, and raspberry kept 79.2%. Light exposure had a clear additive effect on top of

Temperature. At 4 °C, switching from dark to light storage cut blueberry retention from 91.3% to 78.6% at eight weeks - a 12.7 percentage point loss attributable solely to photodegradation. Raspberry suffered the sharpest light penalty: 17.8 points at 25 °C. HPLC profiles showed that delphinidin glycosides in blueberry degraded slowest, while pelargonidin-3-glucoside in raspberry disappeared fastest, losing 68% by week 6 at 25 °C light [12].

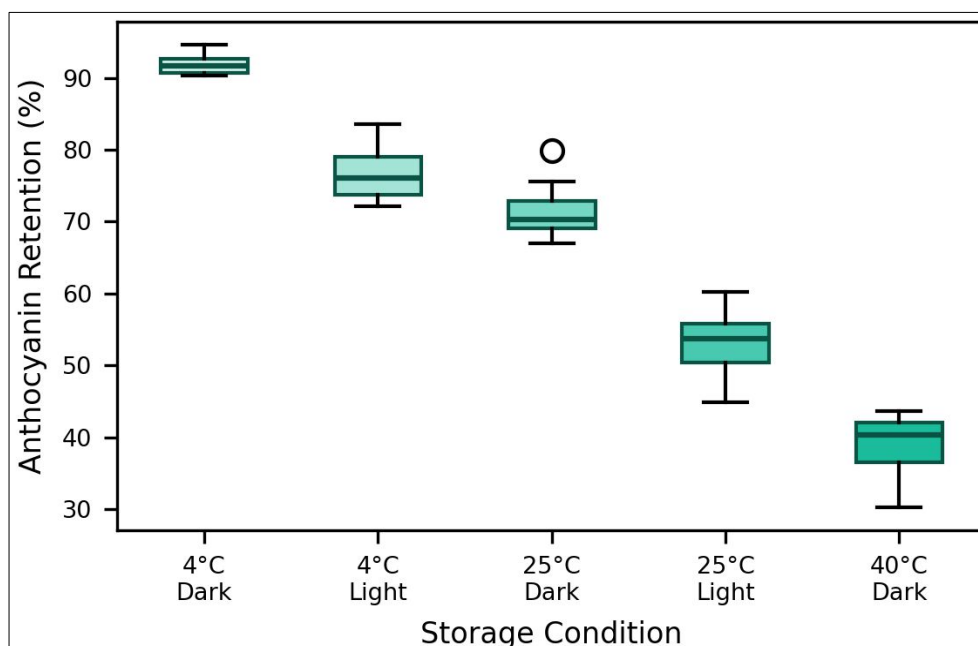


Fig 1: Distribution of anthocyanin retention values across five storage conditions after eight weeks (all three berry types combined).

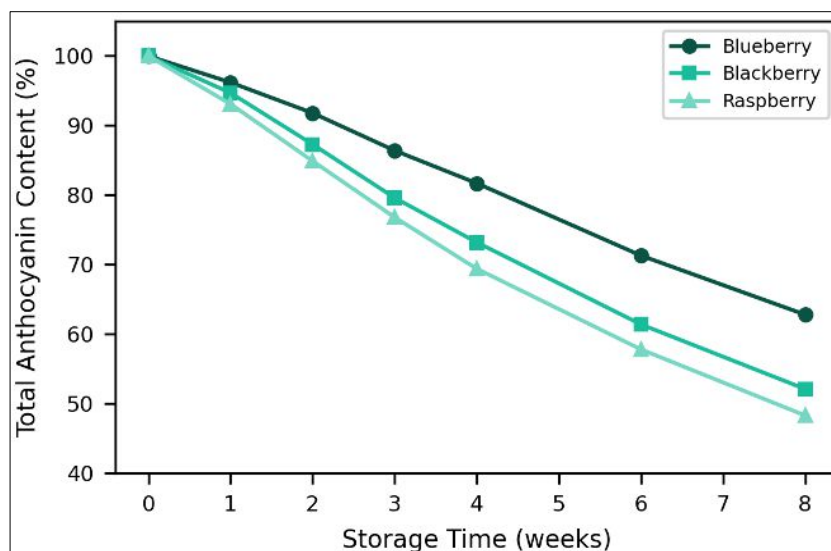


Fig 2: Total anthocyanin content (% of initial) in three berry extracts over eight weeks at 25 °C in the dark.

Discussion

Blueberry's superior stability likely stems from its anthocyanin profile. Malvidin and delphinidin derivatives carry methoxyl groups on the B-ring that shield the flavylium cation from nucleophilic attack, slowing hydrolysis and oxidation compared to the unsubstituted pelargonidin backbone in raspberry [4]. Blackberry sits in the middle, which makes sense given that cyanidin has one hydroxyl group where malvidin has a methoxyl - enough protection to slow things down, but not as much as the fully methylated structure.

The first-order kinetic model fit all degradation curves with R^2 values above 0.96, supporting the idea that anthocyanin breakdown under these conditions follows a simple, concentration-dependent pathway rather than a complex multi-step mechanism. Half-lives at 4 °C dark (blueberry 18.9 weeks, raspberry 12.1 weeks) would translate to roughly 4-5 months of acceptable shelf life for blueberry-based functional beverages stored under proper cold-chain conditions [3]. That's commercially viable, but it drops sharply if temperature control lapses - even one week at 40 °C cut TAC by 28-37% depending on berry type.

Light's contribution was substantial and consistent across berries. Photodegradation proceeds through singlet oxygen generation that attacks the C2-C3 double bond in the anthocyanin chromophore [13]. Packaging in opaque or amber containers is a straightforward mitigation, yet many commercial berry products still use clear bottles or sachets that expose pigments to retail lighting. These data provide concrete numbers to support that packaging choice.

Conclusion

Temperature and light are the two dominant factors controlling anthocyanin survival in berry extracts, with temperature exerting the stronger effect. Blueberry extracts consistently outperformed blackberry and raspberry in retention, a difference traceable to the protective methoxylation of its dominant pigments. Cold, dark storage at 4 °C extended half-lives to nearly 19 weeks for blueberry, making it the most practical candidate for commercial extract production. Formulators working with raspberry or blackberry extracts should anticipate faster degradation and consider encapsulation or copigmentation strategies to compensate.

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