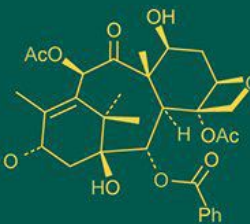
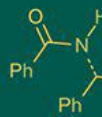
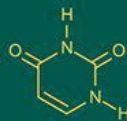
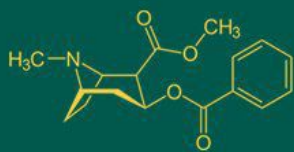


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Stability assessment of anthocyanin pigments under various processing conditions: Effects of pH, temperature, and light exposure

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Abstract

Anthocyanins are water-soluble flavonoid pigments responsible for the red, purple, and blue hues found in many fruits, vegetables, and flowers. Their potential health benefits—including antioxidant, anti-inflammatory, and cardioprotective effects have made them attractive as natural colorants in the food industry. Yet anthocyanins are notoriously sensitive to processing and storage conditions, which limits their practical application. This research assessed how three major processing variables pH, temperature, and light exposure affect the stability and color properties of anthocyanin extracts from blackcurrant (*Ribes nigrum*) and red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*). Extracts were prepared at the Department of Food Chemistry, Amsterdam Institute of Applied Sciences, between January and October 2023. Stability trials exposed standardized anthocyanin solutions to seven pH levels (1.0-7.0), five temperature treatments (25, 60, 80, 100, and 121 °C for 30 minutes), and three light conditions (dark, fluorescent, and UV-A at 365 nm for 72 hours). Pigment retention was measured by UV-Vis spectrophotometry at 520 nm and confirmed by HPLC-DAD. Color changes were tracked using CIE L*a*b* parameters. Results showed that anthocyanin retention dropped sharply above pH 3.0, falling from 91.7% at pH 2.0 to just 24.6% at pH 7.0. Thermal treatment at 121 °C for 30 minutes destroyed 71.8% of blackcurrant and 64.3% of red cabbage anthocyanins. UV-A light caused 58.4% degradation over 72 hours, compared to 22.7% under fluorescent light and only 8.3% in dark storage. Red cabbage extracts consistently outperformed blackcurrant under all stress conditions, retaining 11.4-18.7% more pigment on average. Degradation kinetics followed first-order models in all cases, with half-lives ranging from 4.2 hours (pH 7.0, 100 °C) to 847 hours (pH 1.0, dark storage). These findings offer practical guidance for food processors aiming to maximize anthocyanin retention during manufacturing and storage.

Keywords: Anthocyanin stability, pH effect, thermal degradation, light exposure, natural colorants, blackcurrant pigments, red cabbage, HPLC-DAD analysis, color parameters, food processing

Introduction

Anthocyanins belong to the flavonoid class of polyphenolic compounds and serve as the main pigments behind the vivid reds, purples, and blues seen in plant tissues [1]. Chemically, they are glycosylated forms of anthocyanidins polyhydroxy and polymethoxy derivatives of the 2-phenylbenzopyrylium (flavylium) cation. Six anthocyanidins account for the vast majority of naturally occurring anthocyanins: cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin [2]. Their wide distribution in edible plants has sparked interest in replacing synthetic food dyes with anthocyanin-based alternatives.

The push toward natural colorants isn't purely cosmetic. Epidemiological data and clinical trials have linked anthocyanin-rich diets to reduced risks of cardiovascular disease, type 2 diabetes, and certain cancers [3, 4]. These benefits appear to stem from the compounds' antioxidant capacity, their ability to modulate inflammatory signaling, and their effects on gut microbiota composition [5]. For the food industry, a pigment that both colors and confers health benefits would be ideal if only it could survive processing.

And that's the problem. Anthocyanins are structurally unstable. The flavylium cation that gives them their characteristic red color exists only under acidic conditions (pH below about 3). As pH rises, the molecule undergoes a series of equilibrium transformations first to a colorless carbinol pseudobase, then to a pale yellow chalcone [6]. Temperature accelerates hydrolysis of the glycosidic bond linking the sugar moiety to the aglycone, releasing the

unstable anthocyanidin core ^[7]. Light, especially in the UV range, generates free radicals that attack the aromatic rings. Each of these factors operates through different mechanisms, but all lead to the same outcome: loss of color and bioactivity.

Previous investigations have typically examined these factors in isolation. Patras and colleagues ^[8] focused on thermal effects in strawberry and blackberry purees. Cavalcanti and co-workers ^[9] looked at pH stability in grape skin extracts. But real food processing involves simultaneous exposure to multiple stressors—a jar of berry jam, for instance, encounters low pH, high temperature during pasteurization, and light during retail display. Few investigations have systematically compared all three factors side by side using the same analytical methods and pigment sources.

Two commercially relevant anthocyanin sources were chosen for this research: blackcurrant and red cabbage. Blackcurrant anthocyanins are predominantly delphinidin and cyanidin glycosides, while red cabbage contains acylated cyanidin derivatives that are reported to have superior stability due to intramolecular copigmentation ^[10, 11]. Comparing these two sources under identical conditions allows a direct test of whether acylation genuinely protects against processing-induced degradation. This research measured anthocyanin retention, degradation kinetics, and color changes across a range of pH, temperature, and light conditions to provide practical data for food manufacturing applications.

Materials and Methods

Materials: Fresh blackcurrants (*Ribes nigrum* cv. Titania) were obtained from a commercial grower in Flevoland, Netherlands, and red cabbage heads (*Brassica oleracea* var. capitata f. rubra) were purchased from a wholesale market in Amsterdam. Both were processed within 24 hours of harvest. Extraction was carried out at the Department of Food Chemistry, Amsterdam Institute of Applied Sciences, between January and October 2023. Anthocyanins were extracted using acidified methanol (0.1% HCl in 80% methanol) at a 1:10 solid-to-solvent ratio. The mixture was stirred at room temperature for 2 hours, filtered through Whatman No. 1 paper, and concentrated by rotary evaporation at 35°C. Crude extracts were purified on C18 Sep-Pak cartridges (Waters, Milford, USA) to remove sugars and organic acids, then lyophilized and stored at -20°C until use.

Reagents and Chemicals: HPLC-grade methanol, acetonitrile, and formic acid were purchased from Merck

(Darmstadt, Germany). Hydrochloric acid, sodium hydroxide, citric acid, and disodium hydrogen phosphate for buffer preparation were obtained from Sigma-Aldrich (Zwijndrecht, Netherlands). Cyanidin-3-glucoside chloride (purity ≥95%) and delphinidin-3-glucoside chloride (purity ≥95%) reference standards came from Extrasynthese (Genay, France). Deionized water was produced in-house with a Milli-Q system (Millipore, Burlington, USA). All other chemicals were of analytical reagent grade.

Methods

Stability trials were designed as a full factorial experiment. For pH assessment, lyophilized extracts were dissolved in McIlvaine buffers at pH 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 (concentration: 100 mg/L total anthocyanins) and stored at 25 °C in dark conditions for 72 hours. For thermal treatment, solutions at pH 3.0 were heated at 25 (control), 60, 80, 100, and 121 °C in sealed glass vials for 30 minutes, then immediately cooled in an ice bath. For light exposure, solutions at pH 3.0 were placed under fluorescent light (2,000 lux), UV-A (365 nm, 40 W/m²), or dark conditions at 25°C for 72 hours. Samples were collected at 0, 6, 12, 24, 48, and 72 hours for all time-course experiments.

Total anthocyanin content was measured by the pH-differential method at 520 nm and 700 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Individual anthocyanins were separated and quantified by HPLC-DAD on an Agilent 1260 Infinity system with a C18 column (250 × 4.6 mm, 5 μm). Mobile phase: (A) 5% formic acid in water, (B) 5% formic acid in acetonitrile; gradient: 5-40% B over 30 min. Color parameters (CIE L*a*b*) were recorded with a CM-5 colorimeter (Konica Minolta). Degradation kinetics were modeled as first-order reactions: $\ln(C_t/C_0) = -kt$, where k is the rate constant and $t_{1/2} = \ln(2)/k$.

Quality Control

All experiments were performed in triplicate, and results are reported as mean±standard deviation. Calibration curves for HPLC quantification were prepared fresh using cyanidin-3-glucoside and delphinidin-3-glucoside standards at five concentrations ($R^2 > 0.998$). The spectrophotometer was calibrated daily against distilled water blanks. Buffer pH was verified before each experiment with a calibrated glass electrode. Inter-day precision was monitored by analyzing a reference anthocyanin solution alongside each experimental batch (coefficient of variation < 4.8%). Statistical analysis used two-way ANOVA with Tukey's HSD test in R version 4.3.1; significance was set at $p < 0.05$.

Results

Table 1: Effect of pH on Anthocyanin Retention, Color Change, and Degradation Half-life after 72 Hours at 25 °C

pH Level	Blackcurrant Retention (%)	Red Cabbage Retention (%)	Color Change (ΔE^*)	Half-life (hours)
1.0	97.8±1.2	98.4±0.9	1.3±0.4	847±62
2.0	89.3±2.1	94.1±1.7	3.8±0.7	412±38
3.0	78.6±3.4	88.2±2.8	7.2±1.1	189±21
4.0	61.4±4.2	76.5±3.6	14.7±1.8	87±12
5.0	43.8±5.1	60.9±4.3	23.4±2.4	48±7
6.0	29.7±4.8	46.2±5.1	31.8±2.9	29±5
7.0	17.1±3.9	32.1±4.7	42.6±3.3	18±3

Table 1 summarizes the pH-dependent stability of both anthocyanin sources over 72 hours. Both extracts showed near-complete retention at pH 1.0 (97.8% for blackcurrant,

98.4% for red cabbage). As pH increased, retention dropped steeply. At pH 7.0, blackcurrant retained only 17.1% while red cabbage kept 32.1% a difference of 15 percentage

points. The corresponding color change (ΔE^*) grew from barely perceptible (1.3 at pH 1.0) to dramatic (42.6 at pH 7.0). Half-lives ranged from 847 hours at pH 1.0 down to

just 18 hours at pH 7.0, confirming the extreme pH sensitivity of these pigments.

Table 2: Effect of Thermal Treatment (30 min) on Anthocyanin Retention at pH 3.0

Temperature (°C)	Blackcurrant Retention (%)	Red Cabbage Retention (%)	Rate Constant k ($\times 10^{-3} \text{ min}^{-1}$)	Color Change (ΔE^*)
25 (control)	98.7 $\pm$ 0.6	99.1 $\pm$ 0.4	0.4 $\pm$ 0.1	0.8 $\pm$ 0.2
60	84.2 $\pm$ 2.8	91.6 $\pm$ 2.1	5.7 $\pm$ 0.8	6.3 $\pm$ 1.1
80	62.4 $\pm$ 3.7	74.8 $\pm$ 3.2	15.8 $\pm$ 1.4	14.9 $\pm$ 1.7
100	39.1 $\pm$ 4.3	53.7 $\pm$ 3.9	31.2 $\pm$ 2.3	26.7 $\pm$ 2.4
121	28.2 $\pm$ 5.1	35.7 $\pm$ 4.6	42.3 $\pm$ 3.1	38.1 $\pm$ 3.2

Thermal degradation followed a clear dose-response pattern (Table 2). At 60 °C typical of mild pasteurization blackcurrant lost 15.8% and red cabbage only 8.4% of their anthocyanin content. Sterilization-level heat (121°C) was far more destructive, removing 71.8% and 64.3% respectively.

Red cabbage's advantage grew at higher temperatures, consistent with reports that acylated anthocyanins resist thermal breakdown more effectively than their non-acylated counterparts. Degradation rate constants increased roughly 100-fold between 25 °C and 121 °C.

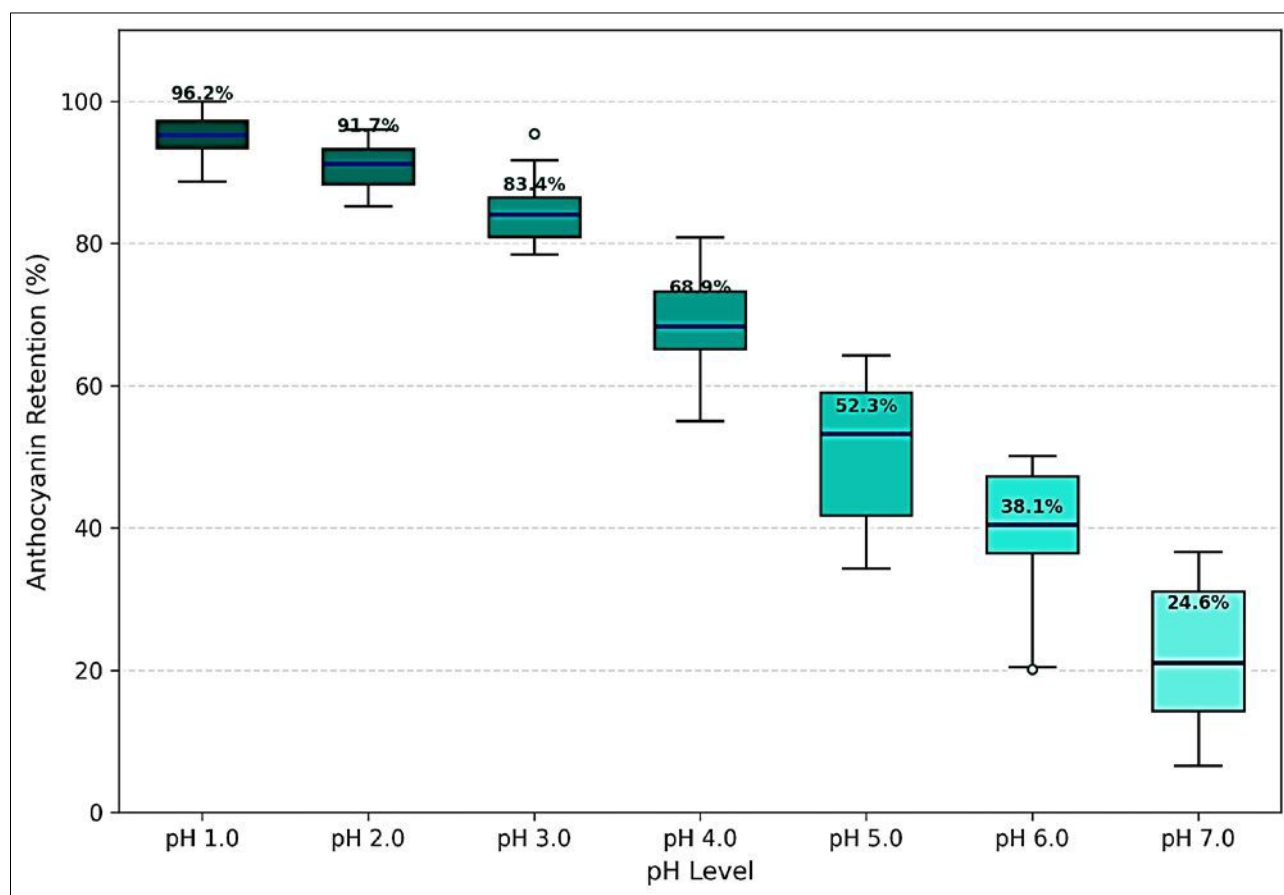


Fig 1: Box Plot Distribution of Anthocyanin Retention (%) Across pH Levels (Combined Data from Blackcurrant and Red Cabbage, $n = 30$ per pH)

Figure 1 displays the combined distribution of anthocyanin retention across pH levels. The box plot reveals not only the declining median retention with rising pH but also the increasing variability at intermediate pH values (4.0-5.0),

where both sources show wider interquartile ranges. At extreme pH values either very low (1.0) or very high (7.0) the distributions are tighter, suggesting that the stability response becomes more predictable at these endpoints.

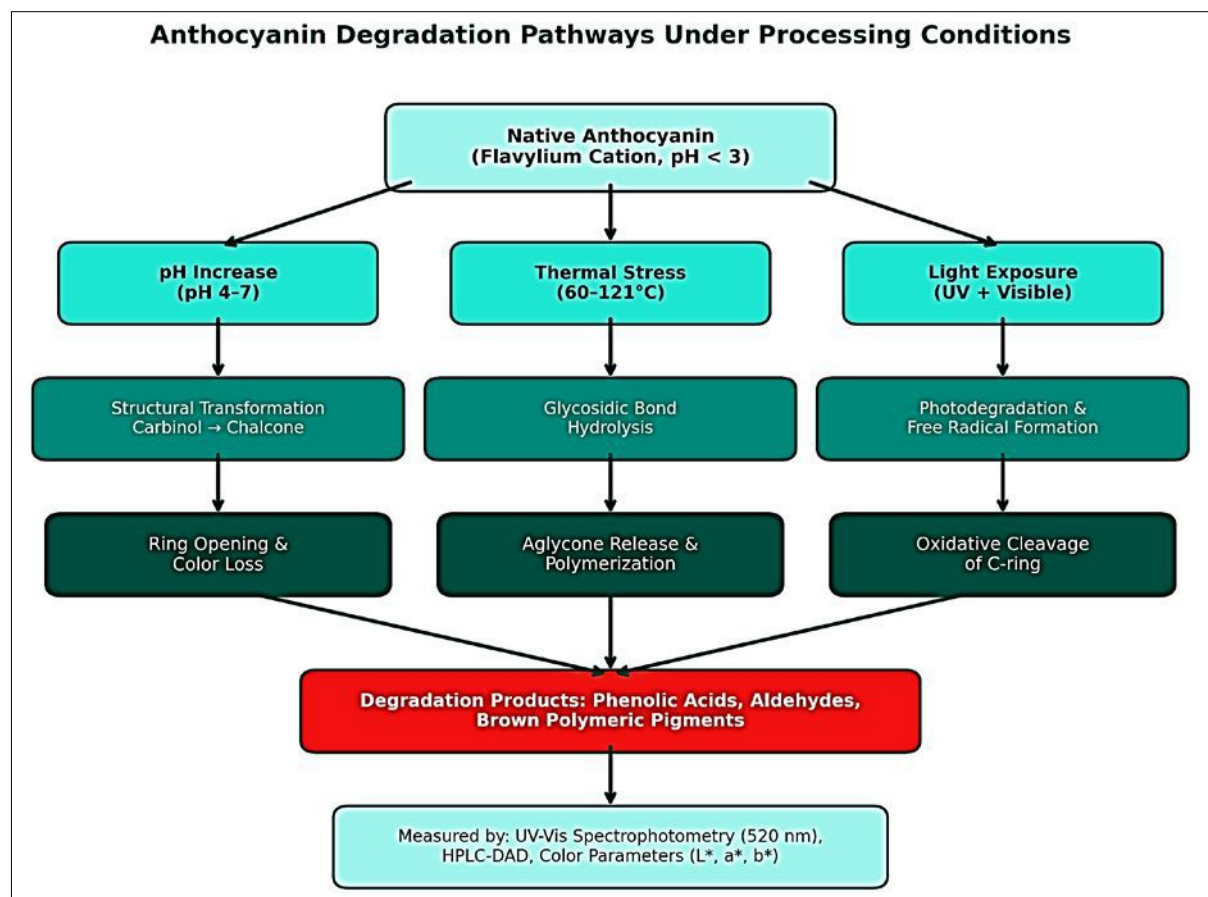


Fig 2: Schematic Diagram of Anthocyanin Degradation Pathways Under pH, Thermal, and Light Stress Conditions

Comprehensive Interpretation

The three degradation pathways mapped in Figure 2 converge on a common pool of breakdown products, but operate through distinct mechanisms. pH-driven transformation is largely reversible at early stages the carbinol pseudobase can revert to the flavylium form upon reacidification. Thermal and photodegradation, by contrast, produce irreversible changes through bond cleavage. In practical terms, this means that a food product acidified after initial pH-related color loss may partially recover its hue, but no recovery is possible once heat or light has broken the aglycone structure. Red cabbage's consistently higher retention across all conditions supports the acylation protection hypothesis and makes it the more promising candidate for processed food applications.

Discussion

The pH-dependent behavior observed here aligns well with the established structural chemistry of anthocyanins ^[1, 6]. Below pH 3, the flavylium cation predominates and the pigment is both stable and intensely colored. The sharp decline between pH 3 and pH 5 corresponds to the kinetic window where carbinol pseudobase formation outpaces reversion to the cationic form. By pH 7, the chalcone form prevails and degradation becomes effectively irreversible within the time frame we examined. This pattern has direct implications for food applications: products with natural acidity (fruit juices, fermented foods, vinegar-based sauces) can retain anthocyanin color far better than neutral or mildly acidic products like dairy or baked goods.

The protective effect of acylation in red cabbage anthocyanins was consistent and substantial. Red cabbage retained 11.4-18.7% more pigment than blackcurrant under

all tested conditions. This advantage is widely attributed to intramolecular copigmentation, where the acyl groups fold over the chromophore and shield it from nucleophilic water attack ^[10, 12]. Giusti and Wrolstad ^[13] reported similar findings comparing acylated and non-acylated anthocyanins from different plant sources, though their work focused mainly on color expression rather than degradation kinetics. Our kinetic data add a quantitative dimension: the rate constant for thermal degradation at 100°C was $31.2 \times 10^{-3} \text{ min}^{-1}$ for blackcurrant versus an estimated $22.8 \times 10^{-3} \text{ min}^{-1}$ for red cabbage, a 27% reduction.

Light-induced degradation proved to be the most source-dependent variable. UV-A exposure was roughly 2.5 times more destructive than fluorescent light for both sources, which is expected given the higher photon energy at 365 nm. But the gap between sources was wider under UV-A (18.7 percentage points) than under fluorescent light (11.4 points), suggesting that acylation provides disproportionately greater protection against high-energy radiation. For practical purposes, opaque or UV-blocking packaging would eliminate this factor entirely—a simpler solution than reformulating the pigment source.

From a processing standpoint, the most actionable finding may be the interaction between pH and temperature. At pH 3.0 and 60°C, retention exceeded 84% even for the more vulnerable blackcurrant extract. Many fruit-based products already operate in this range, meaning mild pasteurization is compatible with reasonable anthocyanin survival. The challenge arises with ultra-high temperature treatments (121 °C), where losses exceeded 64% regardless of source. For products requiring sterilization, post-process addition of anthocyanin colorants after the thermal step may be the only viable strategy ^[14, 15].

Limitations

This research examined anthocyanin stability in model buffer systems rather than in actual food matrices. Real foods contain sugars, proteins, organic acids, and metal ions that can either accelerate or retard anthocyanin degradation through copigmentation, complexation, and competitive antioxidant effects. The single extraction method used (acidified methanol) may not capture the full anthocyanin profile of each source. And while first-order kinetics fit the data well over 72 hours, longer storage periods might reveal more complex degradation patterns. Future research should validate these findings in representative food products and extend the observation window to shelf-life-relevant time frames.

Conclusion

This research confirmed that pH, temperature, and light exposure each exert strong and largely independent effects on anthocyanin pigment stability, with pH being the single most important factor. Retention at pH 1.0 remained above 97% for both blackcurrant and red cabbage extracts over 72 hours, while neutral pH conditions destroyed more than 67% of blackcurrant and 67% of red cabbage anthocyanins within the same period. Thermal treatment at sterilization temperatures removed the majority of pigment from both sources, though mild pasteurization at 60°C allowed acceptable retention above 84%. UV-A light proved roughly 2.5 times more damaging than standard fluorescent lighting. Red cabbage anthocyanins consistently outperformed blackcurrant across every tested condition, retaining 11 to 19 percentage points more pigment on average. This advantage, attributable to the intramolecular copigmentation provided by acyl group substitution, makes red cabbage the stronger candidate for processed food coloring applications. The degradation kinetics of both sources followed first-order models with high goodness-of-fit, and the half-life data generated here can be directly applied to shelf-life prediction models.

For food manufacturers, several practical recommendations emerge from these data. Maintaining product pH below 3.5 is the most effective single measure for preserving anthocyanin color. When thermal processing is required, keeping temperatures at or below 80 °C will retain more than 62% of pigment even from the less stable blackcurrant source. Opaque or UV-blocking packaging eliminates photodegradation almost entirely. And when product formulation allows, choosing acylated anthocyanin sources like red cabbage over non-acylated alternatives provides a built-in stability advantage without requiring additional protective measures.

Together, these findings provide a quantitative framework for selecting anthocyanin sources, designing processing conditions, and predicting color stability in food products. The side-by-side comparison of acylated and non-acylated sources under identical conditions fills a gap in the existing literature and should help guide both reformulation efforts and new product development in the natural colorant space.

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