ASEAN
Journal of Scientific and Technological Reports
Online ISSN:2773-8752

Research article

Effect of Storage Temperature on Degradation and Antioxidant Activity of Anthocyanin in Community–Produced Mao Juice from Sakon Nakhon Province, Thailand

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Citation:
Sripakdee, T.; Roschat, W.; Chaisura, T.; Maiaka, A. Effect of storage temperature on the degradation and antioxidant activity of anthocyanin in community-produced Mao juice from Sakon Nakhon Province, Thailand. *ASEAN J. Sci. Tech. Report.* **2025**, *28*(6), e260077. https://doi.org/10.55164/ajstr.v28i6.260077.

Article history:
Received: June 30, 2025
Revised: September 7, 2025
Accepted: September 12, 2025
Available online: October 14, 2025

Abstract: This study examined the influence of storage temperature and duration on anthocyanin degradation and antioxidant activity in Mao juice, a community-produced beverage from *Antidesma thwaitesianum* in Sakon Nakhon Province, Thailand. Four commercial samples were stored at 4°C and 30°C for 35 days. Anthocyanin content was determined by the pH differential method, while antioxidant activity was assessed using ABTS and FRAP assays. Results showed that Sample A had the highest initial anthocyanin content at 4 °C (41.74 mg/L), decreasing slightly to 40.24 mg/L. At 30 °C, anthocyanin degradation was more pronounced, with Sample A declining from 36.06 to 33.89 mg/L, Sample B from 21.87 to 18.86 mg/L, and Samples C and D dropping from 1.03 to 0.70 mg/L and 0.76 to 0.53 mg/L, respectively. Degradation followed first-order kinetics, with rate constants ranging from 1.0×10^{-3} to 10.9×10^{-3} day^{-1} and half-lives between 99.0 and 9.1 weeks. Sample A was the most stable ($k = 1.0\times10^{-3}$ day^{-1} at 4°C), while Sample C degraded fastest at 30 °C. Antioxidant capacity was highest in Sample A at 4°C on day 0 (1,286.17 mg Trolox/100 mL, ABTS assay), decreasing to 1,240.16 mg by day 35, compared with 1,184.46 mg at 30°C. FRAP values showed parallel trends, with Sample A declining from 826.56 to 811.55 mg Trolox/100 mL at 4°C and from 770.80 to 750.38 mg at 30 °C. The greatest antioxidant loss occurred in Sample B at 30°C ($k = 10.0\times10^{-3}$ day^{-1}). Overall, cold storage was shown to preserve Mao juice stability and functional quality, providing practical guidance for local producers to extend shelf life.

Keywords: Mao juice; anthocyanin degradation; antioxidant activity; storage temperature; community product

1. Introduction

In recent years, a growing global trend toward health consciousness has emerged, particularly through the increasing consumption of natural products. This shift in consumer behavior has led to a substantial expansion of the functional beverage market, with fruit juices playing a prominent role. These beverages are often marketed for their purported health benefits, such as enhancing the immune system, delaying the signs of aging, reducing oxidative stress, and, notably, preventing chronic diseases, including cancer. These health-

promoting effects are primarily attributed to the presence of natural antioxidants found abundantly in fruits and vegetables. Among various antioxidants, anthocyanins have received significant attention due to their dual roles as both pigments and bioactive compounds. Anthocyanins are water-soluble flavonoid compounds responsible for the vivid red, purple, and blue hues in many fruits and vegetables. Their coloration varies with pH, appearing red in acidic environments and shifting to blue under alkaline conditions, making them not only valuable as functional food ingredients but also as natural pH indicators [1-3].

Beyond their aesthetic function, anthocyanins exhibit a range of biological activities, including anti-inflammatory, antimicrobial, and strong antioxidant properties, which contribute to cellular protection against oxidative stress and damage induced by free radicals. These mechanisms are closely linked to the prevention of non-communicable diseases (NCDs), including cardiovascular disease, diabetes, and certain types of cancer. As a result, anthocyanin-rich fruits such as berries, grapes, purple corn, black rice, and purple sweet potatoes have been increasingly incorporated into health-oriented food and beverage products. Ongoing research continues to explore their therapeutic potential, bioavailability, and stability under different processing conditions to enhance their application in functional foods and nutraceuticals [3-6].

Focusing on Sakon Nakhon province, one of the northeastern provinces of Thailand, it is recognized for its rich biodiversity and the presence of many endemic plant species unique to the region. One notable endemic plant is *Antidesma thwaitesianum*, a wild fruit found specifically in Sakon Nakhon. Commonly known as "Mao", this fruit is known to contain a variety of phytochemicals, including polyphenols and anthocyanins, which exhibit strong antioxidant properties [7-9]. In addition to its phytochemical richness, the Mao fruit plays a significant role in local traditions and the local economy. Local communities often process the fruit into ready-to-drink juices, which have become a well-known regional product. Mao–based beverages display a vibrant reddish–purple color due to their high anthocyanin content and are valued for both their flavor and health benefits [10-12]. Scientific studies report that consumption of drinks containing anthocyanin–rich extracts, such as Mao juice can lower postprandial blood glucose levels, suggesting potential applications in glycemic control [13-14]. Furthermore, Mao fruit extracts inhibit monosaccharide–induced protein glycation and suppress the activity of carbohydrate-hydrolyzing enzymes, including alpha-amylase [11, 13, 15-17]. These bioactivities indicate the potential of Mao fruit as a natural functional ingredient for developing products aimed at preventing and managing metabolic disorders, particularly type 2 diabetes. Utilization of Mao fruit in functional beverage development not only enhances the value of local agricultural produce but also provides a promising natural resource for innovations in health–promoting products.

However, the study also reported that the anthocyanins in Mao juice exhibit low stability and are prone to degradation, leading to the formation of colorless compounds. Various factors influence this instability during fruit processing, including temperature, heating duration, and the concentration of soluble solids. In addition to these variables, the storage conditions of anthocyanin–rich fruit juices also play a crucial role in determining the stability of these compounds. Research has shown that the stability of anthocyanins in fruit juices is significantly impacted by both the temperature and the type of fruit juice [18-19]. A study on the stability of anthocyanins in black carrot juice, for example, indicated that temperature fluctuations and the composition of the juice are key determinants of anthocyanin degradation. Furthermore, the degradation of anthocyanin pigments follows first-order kinetic reactions, suggesting a predictable pattern of degradation over time. These findings imply that the antioxidant capacity of Mao juice, along with its anthocyanin content, may alter during storage, especially under suboptimal conditions [20-22]. Such instability presents challenges for the commercialization and long-term storage of anthocyanin-rich products, such as Maqui juice. It is therefore critical to explore methods for enhancing the stability of anthocyanins, such as adjusting processing techniques, optimizing storage conditions, or utilizing stabilizing agents. Improving the shelf life and preserving the bioactive properties of anthocyanin–rich juices would not only enhance their therapeutic potential but also increase their commercial viability. Recent studies have explored several strategies to address these challenges, such as the use of encapsulation techniques, controlled atmosphere packaging, and the addition of natural antioxidants to stabilize anthocyanin pigments. For instance, research conducted by Rezazadeh and Ghasempour [23], Muche et al. [24], and Türkyılmaz and Özkan [25] highlights the role of encapsulation in improving anthocyanin stability in fruit juices under varying storage conditions.

Therefore, the primary objective of this study is to investigate the impact of temperature and storage time on the anthocyanin content and antioxidant capacity in Mao juice, a locally produced beverage from Sakon Nakhon Province. This research explicitly examines how varying temperatures affect the degradation rates of anthocyanins and antioxidants in Mao juice, as well as the influence of storage time on these bioactive compounds. The study focuses on evaluating the effect of temperature on anthocyanin degradation over 35 days, with samples stored under two conditions: room temperature (30 °C) and refrigeration (4 °C). These temperatures were selected to reflect common storage conditions, with 4°C representing typical refrigeration that slows microbial growth and chemical degradation, and 30 °C representing ambient conditions that the juice may encounter during transportation or storage without cooling, allowing for a comparison of anthocyanin stability under both conditions. Anthocyanin content is quantified using the pH Differential method, while antioxidant activity is assessed using the ABTS and FRAP assays. Additionally, the study tracks the rate of degradation of both anthocyanins and antioxidants over the storage period. The findings from this study are expected to provide valuable insights for local community-based Mao juice producers. The results will help identify key production and storage factors that influence the retention of vital compounds in the juice, ultimately offering guidance on best practices for maintaining product quality and optimizing the health benefits for consumers.

2. Materials and Methods

2.1 Materials

This study investigated four Mao juice samples, designated as Sample A, B, C, and D, sourced from different commercial brands in Sakon Nakhon Province, to evaluate the effects of storage temperature on anthocyanin degradation and antioxidant activity. Brand names have been omitted and replaced with sample codes to avoid potential commercial implications, as the findings regarding bioactive compound levels may influence consumer purchasing decisions and, consequently, impact the reputation or sales of the producers. To maintain experimental accuracy and reliability, a variety of chemicals were used. The key reagents included ethanol (C_2H_5OH), which was utilized for extraction; hydrochloric acid (HCl) and sodium acetate (CH_3COONa) for pH adjustments during the analysis; potassium chloride (KCl) and aluminum chloride ($AlCl_3$) for preparing various assay solutions; and antioxidants such as 2,2′-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), employed to assess antioxidant capacity. Additionally, potassium persulfate was used in the preparation of oxidizing agents. All chemicals were sourced from reputable suppliers, including Sigma–Aldrich, Fluka, QRëC, Merck, Acros Chemical Co. Ltd., and Carlo Erba, ensuring that high-quality reagents were utilized for precise and consistent experimental outcomes.

2.2 Sample preparation

Four commercially available Mao juice samples, designated as Samples A, B, C, and D, were utilized in this study. Each brand provided 500 mL of freshly produced juice, prepared in triplicate. The samples were stored at two different temperatures: refrigeration (4 °C) and ambient room temperature (30 °C). To prevent light-induced degradation, all sample bottles were wrapped in aluminum foil and stored in a dark environment. These temperatures were chosen to reflect common storage conditions: 4°C represents typical refrigeration, which slows microbial growth and chemical degradation, while 30 °C represents ambient conditions that the juice may encounter during transportation or storage without refrigeration. This setup enabled the evaluation of juice stability under both refrigerated and room-temperature conditions. Analyses were conducted over 35 days, with sampling on days 0 (baseline), 7, 14, 21, 28, and 35. At each sampling interval, the juice samples underwent filtration to remove pulp and particulate matter, followed by centrifugation at 3,000 rpm for 20 minutes to separate the sediments. The resulting supernatant was collected for subsequent analyses. For the extraction of anthocyanins and antioxidants, 1 mL of the clarified juice was mixed with 2 mL of ethanol acidified with 1% hydrochloric acid (v/v), achieving a 1:2 sample–to–solvent ratio. The mixture was incubated at room temperature for 2 h in the absence of direct sunlight to prevent degradation of sensitive compounds. Post–incubation, samples were centrifuged at 4,000 rpm for 10 min, and the clear supernatant was collected for the determination of anthocyanin content and antioxidant capacity.

This methodology aligns with established protocols for anthocyanin and antioxidant extraction, ensuring the stability and integrity of bioactive compounds during analysis [26-27].

2.3 Determination of anthocyanin content by the pH differential method

Total anthocyanin content (TAC) was determined using the pH differential method, following the procedures described by Chua et al. [28-9] and Handayani et al. [30]. This method is based on the principle that anthocyanin structures undergo reversible changes in response to pH variation, thereby altering their absorbance properties. In this technique, anthocyanin extracts were adjusted to pH levels of 1.0 and 4.5, and their absorbance was measured across a wavelength range of 260-700 nm. Anthocyanins typically exhibit maximum absorbance between 460 and 560 nm. At pH 1.0, anthocyanins predominantly exist in the colored oxonium form, resulting in high absorbance within this range. In contrast, at pH 4.5, anthocyanins transition to the colorless hemiketal form, leading to a loss of absorbance at these wavelengths. If other compounds that absorb at similar wavelengths are present, their absorbance values remain unchanged under both pH conditions. Thus, the absorbance difference between pH 1.0 and pH 4.5 is explicitly attributed to anthocyanins. To correct for sample turbidity or light scattering, absorbance at 700 nm was also recorded at both pH levels and subtracted from the measurements. Finally, the total anthocyanin content was calculated based on the corrected absorbance values using the appropriate formula, ensuring greater accuracy and precision of the results. The total anthocyanin content (TAC) in Mao juice samples was determined using the pH–differential method, adapted from the procedures described by Chua et al. [28-29] and Handayani et al. [30]. Initially, the Mao juice extracts were diluted 100-fold with distilled water to ensure that the absorbance readings fell within the optimal range for accurate measurement. The final volume of each diluted sample was adjusted to 100 mL. A preliminary absorbance measurement at 520 nm was conducted using a UV-Visible spectrophotometer to verify that the absorbance was within the desirable range of 0.2-0.8, which minimizes errors associated with instrumental sensitivity. Following the preliminary check, the diluted samples were prepared for pH–differential analysis by mixing 2 mL of the sample with 2 mL of 0.025 M potassium chloride buffer solution at pH 1.0 to form the first mixture. Similarly, 2 mL of the sample was mixed with 2 mL of 0.4 M sodium acetate buffer solution at pH 4.5 to form the second mixture. Both mixtures were allowed to equilibrate at room temperature for 15 min, protected from direct sunlight to prevent anthocyanin degradation.

After equilibration, the absorbance of each mixture was measured at two specific wavelengths: 520 nm, corresponding to the maximum absorption peak of anthocyanins, and 700 nm, used to correct for any turbidity or light scattering. Distilled water was used as the blank for all spectrophotometric measurements. The absorbance values were recorded separately for the mixtures at pH 1.0 and pH 4.5. The net absorbance (A) related to anthocyanin concentration was calculated using the following formula (1):

$$A = (A_{520} - A_{700})_{pH\ 1.0} - (A_{520} - A_{700})_{pH\ 4.5} \quad (1)$$

where A_{520} and A_{700} represent the absorbance values at 520 nm and 700 nm, respectively, under each pH condition. The monomeric anthocyanin content, expressed as milligrams of cyanidin-3-glucoside equivalents per liter (mg/L), was calculated according to the following equation (2):

$$\text{Monomeric anthocyanin (mg/L)} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l} \quad (2)$$

Where MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF is the dilution factor (i.e., the ratio of the final volume to the initial volume of the diluted sample), ϵ is the molar absorptivity coefficient of cyanidin-3-glucoside in a pH 1.0 buffer (26,900 L/mol cm), and l is the path length of the cuvette (1 cm). As shown in Figure 1, the experimental procedure for determining anthocyanin content was carried out using the pH differential method. All measurements were performed in triplicate for each sample to ensure the precision and reliability of the results.

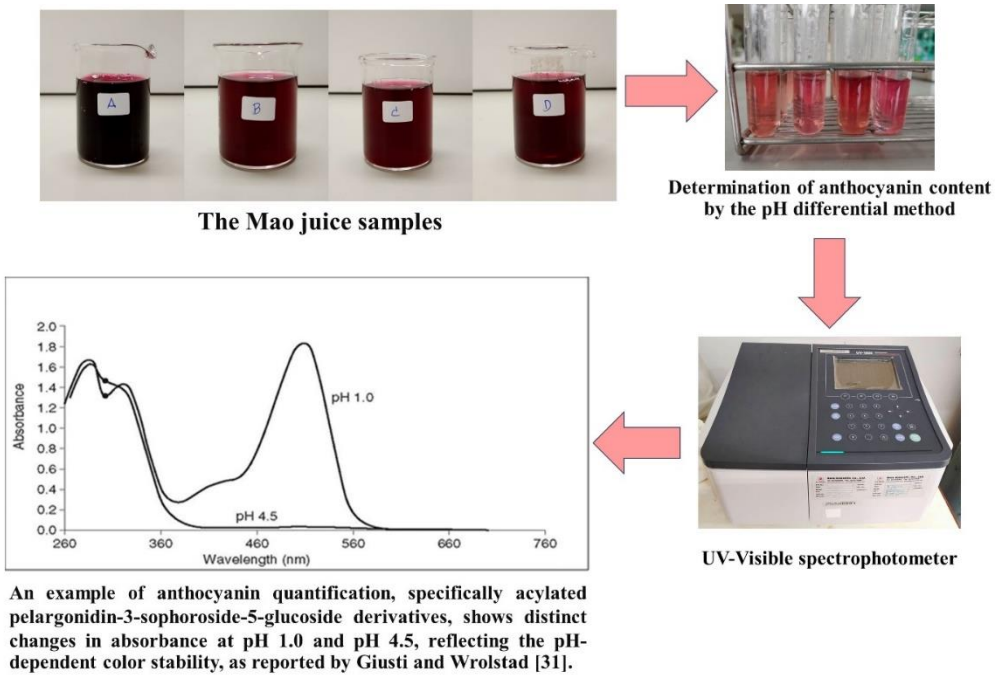

Figure 1. The experimental procedure for determining anthocyanin content using the pH differential method.

2.4 Antioxidant activity analysis

2.4.1 ABTS radical scavenging assay

The in vitro antioxidant activity was evaluated using a modified ABTS radical scavenging assay, following the methods described by Li et al. [31], Cai et al. [32], and Bai et al. [33]. The experimental procedure commenced with the collection of the Mao juice samples stored at refrigeration temperature (4 °C) and ambient room temperature (30 °C) on days 0 (baseline), 7, 14, 21, 28, and 35. The samples were prepared according to the method described in Section 2.2. For the ABTS radical scavenging assay, 7 mM ABTS stock solution and 2.45 mM potassium persulfate solution were prepared separately. Equal volumes of the two solutions were mixed and allowed to react in the dark at room temperature for 12–16 h to generate the $ABTS^{\bullet+}$ radical cation. The resulting $ABTS^{\bullet+}$ solution was subsequently diluted with ethanol to achieve an absorbance of 1.00 ± 0.02 at 734 nm. For the antioxidant assay, the Mao juice samples were diluted 500-fold and 100-fold with distilled water, depending on the expected antioxidant activity. An aliquot of 1 mL of the diluted sample was mixed with 3 mL of the prepared $ABTS^{\bullet+}$ working solution. After incubation at room temperature for 6 min in the dark, the absorbance at 734 nm was measured using a UV–Visible spectrophotometer (UV-1800, Shimadzu, Japan). Ethanol was used as a blank control. As displayed in Figure 2, the antioxidant activity of Mao juice samples was analyzed using a modified ABTS radical scavenging assay. The antioxidant capacity of each sample was quantified by comparing the percentage inhibition of $0ABTS^{\bullet+}$ with a standard curve of Trolox and expressed as milligrams of Trolox equivalents per 100 mL of sample (mg TE/100 mL). All measurements were performed in triplicate to ensure accuracy and reproducibility. This technique offers several advantages, including its ability to measure both hydrophilic and lipophilic antioxidant activity. It is also susceptible, rapid, and adaptable for use with a wide variety of sample types, including fruit juices. These strengths make the ABTS assay a robust and reliable method for antioxidant evaluation in complex food matrices.

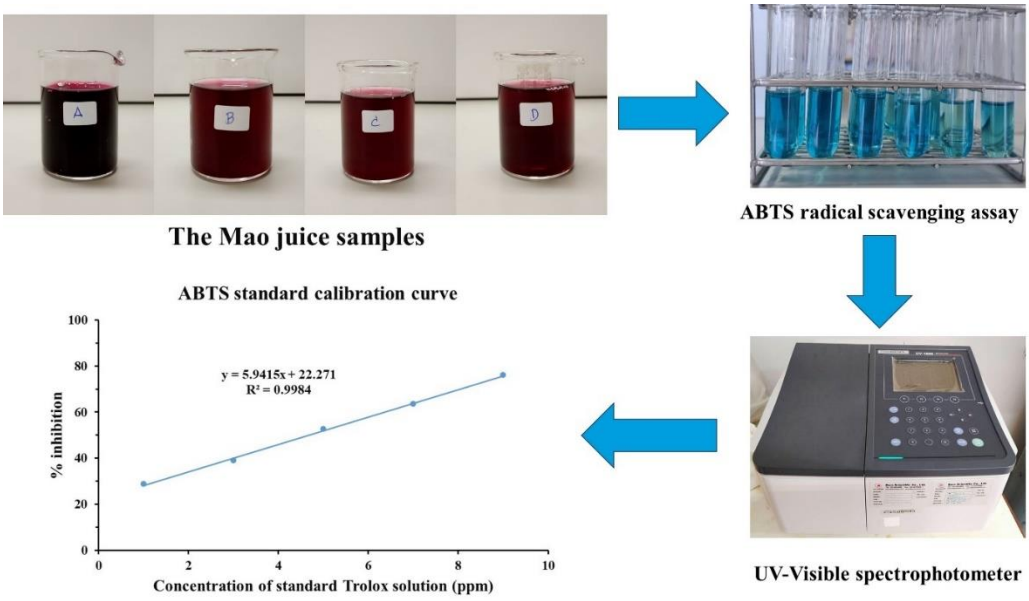

Figure 2. Analysis of antioxidant activity in Mao juice samples using a modified ABTS radical scavenging assay.

2.4.2 Ferric Reducing Antioxidant Power (FRAP) assay

The antioxidant capacity of Mao juice samples was evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay, adapted with modifications based on the methods reported by Bamigbade et al. [34], Spiegel et al. [35], and Muthu et al. [36]. The FRAP reagent was freshly prepared before each analysis by mixing three solutions in the ratio of 10:1:1. These included (1) 300 mM acetate buffer (pH 3.6), (2) 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution dissolved in 40 mM hydrochloric acid, and (3) 20 mM ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) solution. The mixed reagent was pre-warmed to 37 °C in a water bath prior to use. For the calibration curve, 1 mL of Trolox standard solution at various concentrations was mixed with 3 mL of the FRAP reagent. The mixture was incubated at 37 °C for 10 minutes, and the absorbance was measured at 593 nm using a UV-visible spectrophotometer. A standard curve was constructed by plotting absorbance versus Trolox concentration (Figure 3) and used to determine the antioxidant capacity of Mao juice samples. The FRAP assay offers several advantages, including simplicity, rapidity, and low cost, making it ideal for routine antioxidant screening in food samples. It also measures the reducing ability of antioxidants, which is closely associated with their capacity to donate electrons—a fundamental mechanism of antioxidant action. Furthermore, the assay demonstrates high compatibility with aqueous extracts, making it suitable for evaluating fruit juices and other hydrophilic food matrices. The Mao juice samples, obtained from the extraction procedure described in Section 2.2, were initially diluted 100-fold with distilled water to ensure that the absorbance readings would fall within the linear range of the FRAP assay. An aliquot of 1 mL of each diluted extract was thoroughly mixed with 3 mL of the freshly prepared FRAP reagent. The mixture was incubated at 37 °C for 10 min to allow complete reaction, and the absorbance was subsequently measured at 593 nm using a UV-Visible spectrophotometer. Ethanol or distilled water was used as a blank control to calibrate the instrument prior to sample measurements. The antioxidant capacity was expressed as milligrams of Trolox equivalents per 100 mL of sample (mg TE/100 mL), based on interpolation from the Trolox calibration curve. All measurements were performed in triplicate to ensure accuracy and reproducibility.

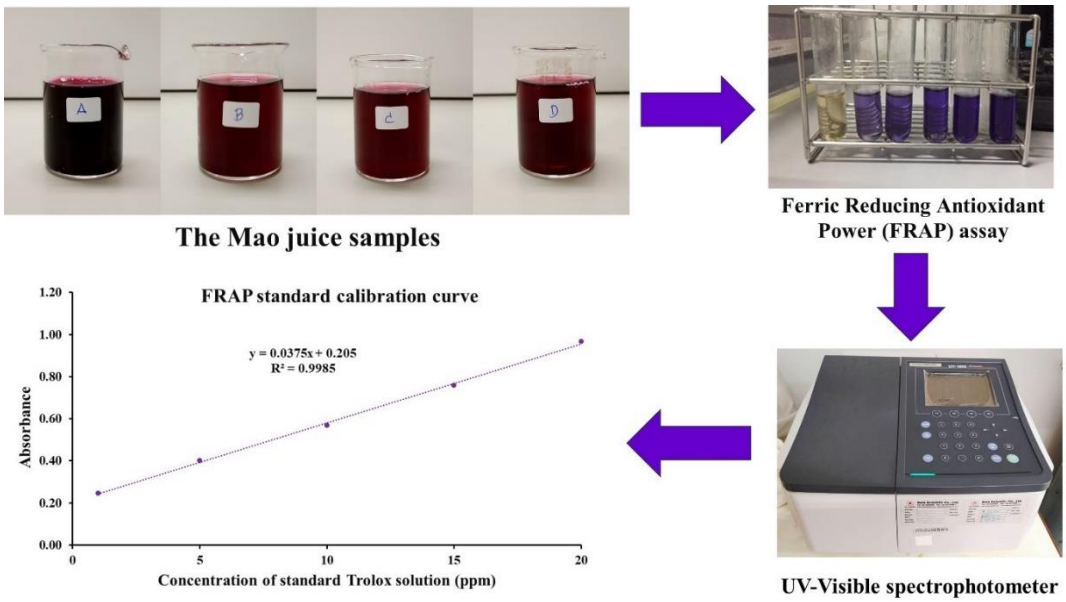

Figure 3. Determination of the antioxidant capacity of Mao juice samples using the Ferric Reducing Antioxidant Power (FRAP) assay.

2.4.3 Degradation kinetics and stability of anthocyanins and antioxidant activity in Mao juice

The degradation kinetics and stability of anthocyanins in Mao juice were investigated by monitoring samples stored at two temperature conditions—refrigeration (4 °C) and ambient room temperature (30 °C)—over a 35-day storage period. Mao juice samples were collected on days 0, 7, 14, 21, 28, and 35, and the total anthocyanin content (TAC) was quantified using the pH differential method, as described in Section 2.2. To evaluate the degradation behavior, a first-order kinetic model was applied, as described in the research reports of Hernandez (Prieto et al. [37] and Chen et al. [38]). The natural logarithm of the ratio of anthocyanin concentration at time (t) to the initial concentration, $\ln(C_t/C_0)$, was plotted against storage time (t), as shown in the following equation (3):

$$\ln(C_t/C_0) = -kt \quad (3)$$

Where C_t is the anthocyanin concentration at time (t), C_0 is the initial anthocyanin concentration, k is the first-order rate constant, and t is the storage time. A linear relationship between $\ln(C_t/C_0)$ and t confirmed that the degradation of anthocyanins followed first-order kinetics. The degradation rate constant (k) was calculated from the slope of the linear regression. The half-life ($t_{1/2}$) of anthocyanins was then determined using equation (3):

$$t_{1/2} = \frac{-\ln 0.5}{k} \approx \frac{0.693}{k} \quad (4)$$

This kinetic approach is widely used for evaluating the thermal sensitivity and oxidative degradation of anthocyanins in fruit-based products. It provides valuable insight into the stability of bioactive compounds under different storage conditions, facilitating optimization of product shelf life and quality preservation strategies.

2.5 Statistical Analysis

All experimental measurements were performed in triplicate, and results are presented as mean ± standard deviation (SD). The data in this study were reported as averages from repeated experiments, and both the standard deviation and percent error were taken into account. All results showed a percent error not exceeding 3%, indicating acceptable precision of the measurements. As this research represented a preliminary study, the four Mao juice samples (Samples A, B, C, and D) were obtained from different commercial brands in Sakon Nakhon Province, each with distinct production processes. Therefore, the study did not emphasize

detailed statistical comparisons among brands; instead, it focused on overall trends in the degradation of anthocyanin content—the principal bioactive compound—and on changes in antioxidant activity. In addition, degradation kinetics and stability analyses were employed to describe the behavior of anthocyanins and their antioxidant activity during storage at various temperatures, and the results were interpreted using basic statistical methods for experimental estimation.

3. Results and Discussion

3.1 Determination of total anthocyanin content using the pH differential method

The total anthocyanin content (TAC) of Mao juice from four commercial brands was monitored during storage at refrigeration temperature (4 °C) and ambient temperature (30 °C) using the pH differential method, with quantification based on comparison to a cyanidin-3-3-glucoside standard. As presented in Table 1, Sample A exhibited the highest initial anthocyanin concentration, followed by Samples B, C, and D, respectively. These differences are likely attributable to variability in raw material selection, cultivar types, harvest maturity, and post-harvest handling. Furthermore, processing methods such as pasteurization, extraction, and filtration may play a significant role in preserving or degrading heat-sensitive compounds, including anthocyanins. Storage temperature was found to have a notable influence on anthocyanin stability. A higher storage temperature (30 °C) accelerated the degradation of anthocyanins across all samples compared to refrigeration at 4 °C. This observation aligns with the known thermal sensitivity of anthocyanin pigments, which are susceptible to structural transformation and oxidation at elevated temperatures [4,27,37-40]. In addition, the anthocyanin content exhibited a gradual decline over time under both storage conditions, with a more pronounced decrease at 30 °C. The degradation pattern observed suggests that prolonged exposure to elevated temperatures exacerbates anthocyanin breakdown, possibly due to enhanced kinetic activity leading to hydrolysis and polymerization reactions. These results indicate that both temperature and storage duration are critical factors in maintaining anthocyanin stability in Mao juice, underscoring the importance of cold chain storage for extending shelf life and preserving the functional quality of anthocyanin-rich beverages.

Table 1. Total anthocyanin content (mg/L) of Mao juice from four commercial brands during storage at 4 °C and 30 °C.

Sample of Mao juice	Storage temperature	Total anthocyanin content (mg/L)					
		Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
Sample A	4 °C	41.74	41.41	41.07	40.91	40.57	40.24
Sample B	4 °C	29.89	29.22	28.72	28.38	28.05	27.88
Sample C	4 °C	1.26	1.20	1.16	1.13	1.10	1.07
Sample D	4 °C	1.03	1.00	0.96	0.90	0.85	0.81
Sample A	30 °C	36.06	35.40	34.90	34.56	34.06	33.89
Sample B	30 °C	21.87	21.20	20.70	20.20	19.53	18.86
Sample C	30 °C	1.03	0.96	0.86	0.84	0.76	0.70
Sample D	30 °C	0.76	0.73	0.70	0.66	0.56	0.53

3.2 Degradation kinetics and stability of anthocyanins in Mao juice

The degradation behavior of anthocyanins in Mao juice under various storage conditions was analyzed by monitoring changes at 4 °C and 30 °C over 35 days. Figure 4 illustrates the linear relationship between the natural logarithm of the anthocyanin content ratio ($\ln(C_t/C_0)$) and storage time (t), confirming that anthocyanin degradation follows first-order reaction kinetics across all samples. The strong linearity (R^2 values approaching 1.0) at both temperatures further supports this kinetic model. From Figure 4, it is evident that the slope of the degradation curves increases with temperature, indicating a higher degradation rate at 30 °C compared to 4 °C. This trend is quantified in Table 2, where all samples exhibit higher degradation rate constants (k) and shorter half-lives ($t_{1/2}$) at elevated temperature. The thermal acceleration of anthocyanin breakdown aligns with known degradation mechanisms involving enhanced molecular mobility and oxidative reactions under heat.

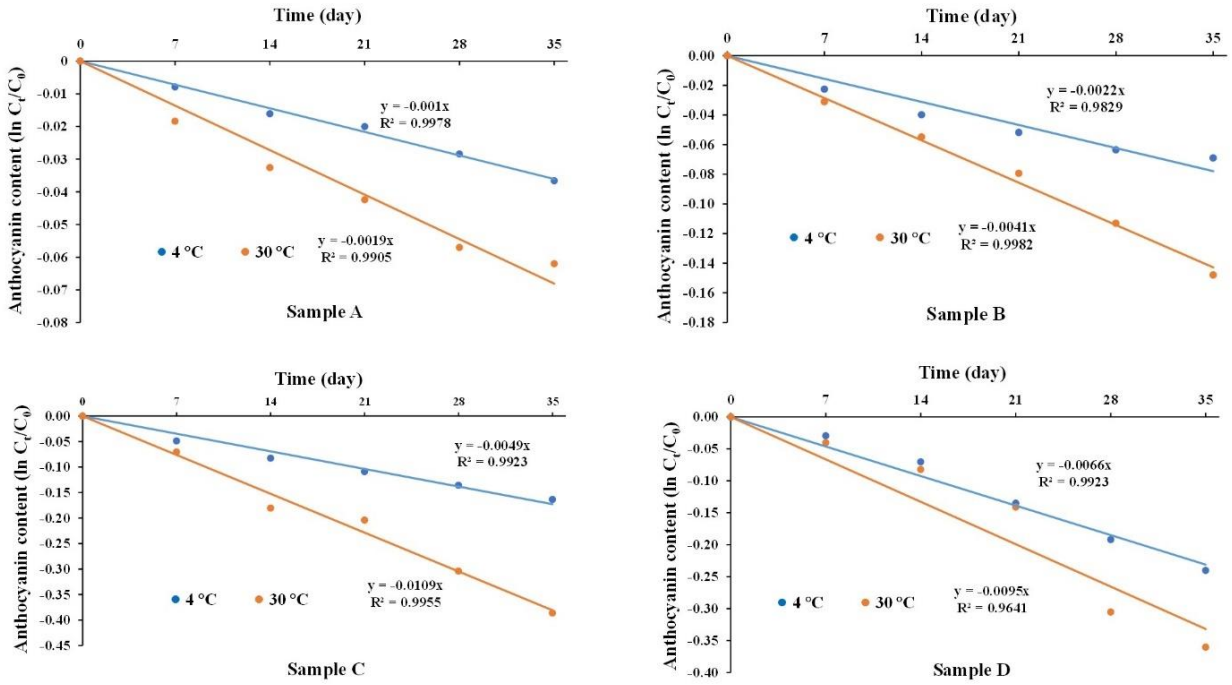

Figure 4. Degradation rate of anthocyanins in Mao juice stored at 4 °C and 30 °C over 35 days, analyzed using the pH–differential method.

Table 2. Degradation kinetics and half-life ($t_{1/2}$) of anthocyanins in Mao juice during storage at 4 °C and 30 °C, determined by the pH–differential method assuming first-order kinetics.

Sample of Mao juice	Storage temperature	Degradation rate constant k × 10^{-3} (day^{-1})	Half–life ($t_{1/2}$) (week)
Sample A	4 °C	1.0	99.0
Sample B	4 °C	2.2	45.0
Sample C	4 °C	4.9	20.2
Sample D	4 °C	6.6	15.0
Sample A	30 °C	1.9	52.1
Sample B	30 °C	4.1	24.1
Sample C	30 °C	10.9	9.1
Sample D	30 °C	9.5	10.3

Among the four samples, Sample A showed the most significant resistance to anthocyanin degradation, with the lowest k values (1.0 × 10^{-3} day^{-1} at 4 °C and 1.9 × 10^{-3} day^{-1} at 30 °C) and the most prolonged half-lives (99.0 and 52.1 weeks, respectively). This suggests that Sample A may contain more stable anthocyanin structures or protective components such as co-pigments or antioxidants. In contrast, Samples C and D exhibited significantly higher degradation rates at 30 °C (k = 10.9 × 10^{-3} and 9.5 × 10^{-3} day^{-1}) and shorter half-lives (9.1 and 10.3 weeks), highlighting their lower anthocyanin stability under ambient conditions. These differences may result from variations in juice composition, pH, enzymatic activity, or the presence of prooxidant compounds [5, 21, 32, 38-40]. These results highlight the critical role of low–temperature storage in preserving anthocyanin stability and extending the shelf life of Mao juice, in agreement with previous research on thermally sensitive bioactive compounds in fruit-based beverages.

3.3 Changes in antioxidant activity of Mao juice during storage

3.3.1 Effect of temperature and storage duration on the degradation rate of antioxidants in Mao juice using the ABTS method

The antioxidant activity of Mao juice stored at different temperatures was assessed using the ABTS radical cation decolorization assay, as displayed in Table 3. The results clearly demonstrated a time-dependent and temperature-sensitive decline in antioxidant capacity over a 35-day storage period. Samples stored at 30 °C exhibited a more rapid reduction in antioxidant activity compared to those stored at 4 °C, indicating that elevated temperatures accelerate oxidative degradation of phenolic compounds, including anthocyanins and other antioxidant constituents. Among the tested samples, Sample A consistently showed the highest antioxidant levels throughout the study, suggesting superior compositional or varietal stability. In contrast, Sample D exhibited the lowest antioxidant activity, particularly under higher temperature conditions, reflecting greater susceptibility to oxidative deterioration. The ABTS assay was selected for this study due to its sensitivity, reproducibility, and broad applicability in determining the total antioxidant capacity of complex mixtures. One of its key advantages lies in its ability to measure both hydrophilic and lipophilic antioxidant compounds, providing a comprehensive assessment of antioxidant potential. Moreover, the ABTS radical is stable in aqueous and organic solvents and can be generated enzymatically or chemically, making it suitable for routine analysis of food and beverage matrices. The findings underscore the critical role of cold storage (e.g., 4°C) in maintaining the antioxidant integrity of Mao juice, thereby helping to preserve its functional quality and extend its shelf life [31, 33, 41-42].

Table 3. Antioxidant content determined by the ABTS method in Mao juice stored at 4 °C and 30 °C over the storage period.

Sample of Mao juice	Storage temperature	Antioxidant content (mg Trolox / 100 mL)					
		Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
Sample A	4 °C	1286.17	1279.10	1263.85	1253.59	1248.01	1240.16
Sample B	4 °C	162.04	159.81	158.48	156.35	155.71	153.61
Sample C	4 °C	147.56	147.09	145.68	143.05	140.95	139.85
Sample D	4 °C	125.21	124.33	123.11	122.63	120.01	118.24
Sample A	30 °C	1246.78	1235.36	1224.59	1214.30	1202.38	1184.46
Sample B	30 °C	150.19	147.36	146.06	143.86	142.22	140.52
Sample C	30 °C	137.73	136.18	132.98	130.15	129.14	124.15
Sample D	30 °C	124.60	122.99	119.89	116.99	115.72	113.68

The degradation kinetics of antioxidants in Mao juice, as evaluated by the ABTS method under the assumption of first-order kinetics, provide significant insights into the stability of antioxidant compounds during storage at two different temperatures: 4 °C and 30 °C. The degradation rate constants (k) and corresponding half-lives ($t_{1/2}$) presented in Table 4 and Figure 5 demonstrate the impact of storage temperature and sample variation on antioxidant stability. At 4 °C, all samples exhibited relatively lower degradation rate constants, ranging from 1.0 to 1.6 × 10^{-3} day^{-1}, corresponding to half-lives of approximately 62 to 99 weeks. Sample A exhibited the slowest degradation rate (k = 1.0 × 10^{-3} day^{-1}) and, consequently, the longest half-life (99 weeks), indicating superior antioxidant stability under refrigeration. Samples B, C, and D exhibited similar degradation rates (1.5-1.6 × 10^{-3} day^{-1}), resulting in shorter half-lives of approximately 62 to 66 weeks. This suggests minor variations in antioxidant composition or initial concentration among the samples, possibly due to differences in raw material quality, processing, or formulation.

Table 4. Degradation rate constants and half-life ($t_{1/2}$) of antioxidants in Mao juice during storage at 4 °C and 30 °C, determined by the ABTS method assuming first-order kinetics.

Sample of Mao juice	Storage temperature	Degradation rate constant $k \times 10^{-3}$ (day^{-1})	Half–life $t_{1/2}$ (week)
Sample A	4 °C	1.0	99.0
Sample B	4 °C	1.6	61.9
Sample C	4 °C	1.5	66.0
Sample D	4 °C	1.6	61.9
Sample A	30 °C	1.4	70.7
Sample B	30 °C	1.9	52.1
Sample C	30 °C	2.7	36.7
Sample D	30 °C	2.7	36.7

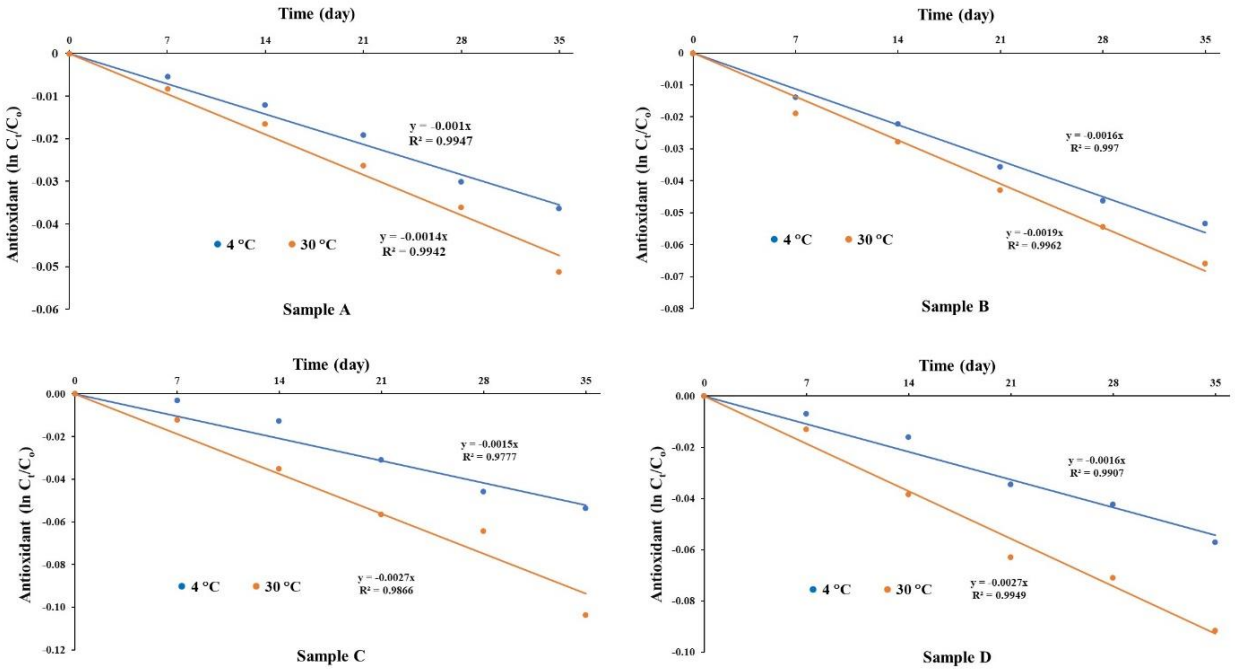

Figure 5. Degradation rate of antioxidants in Mao juice stored at 4 °C and 30 °C over 35 days, determined by the ABTS method.

When storage temperature increased to 30 °C, the degradation rates accelerated markedly, with k values increasing to between 1.4 and 2.7 × 10^{-3} day^{-1}. Correspondingly, the half-life shortened significantly to between 37 and 71 weeks. Sample A again demonstrated relatively better stability at elevated temperature with a degradation rate constant of 1.4 × 10^{-3} day^{-1} and a half–life of approximately 71 weeks, although this stability was noticeably reduced compared to storage at 4 °C. Samples C and D were the most susceptible to degradation at 30 °C, both exhibiting the highest rate constants (2.7 × 10^{-3} day^{-1}) and the shortest half–lives (36.7 weeks). This increase in degradation rate with temperature aligns well with the general understanding that antioxidant compounds are thermally labile and undergo accelerated oxidative degradation under higher thermal conditions [25, 37-38, 40-41]. The findings suggest that refrigeration significantly prolongs the antioxidant activity in Mao juice, potentially extending its functional shelf life. Conversely, storage at ambient or elevated temperatures compromises the antioxidant stability of the juice, potentially reducing its antioxidant effectiveness and overall quality. Moreover, the variability among samples highlights the importance of consistent raw material selection and processing controls to optimize antioxidant retention.

Therefore, controlling storage temperature is crucial for preserving antioxidant potency in Mao juice. The observed first-order degradation kinetics provide a helpful model for predicting shelf life under various storage conditions, which is valuable for both producers and consumers aiming to maximize the health benefits associated with antioxidants in Mao juice.

3.3.2 Effect of temperature and storage duration on the degradation rate of antioxidants in Mao juice using the FRAP assay

The results obtained from the FRAP assay, as presented in Table 5, clearly demonstrate that the antioxidant content in Mao juice declined over time at both storage temperatures, with a more pronounced degradation observed at 30 °C. Sample A, which initially exhibited the highest antioxidant capacity at 4 °C (826.56 mg Trolox/100 mL), showed a gradual decrease throughout the 35-day storage period, dropping to 811.55 mg Trolox/100 mL. In contrast, at 30 °C, the antioxidant content in the same sample started at a lower level (770.80 mg Trolox/100 mL) and declined more rapidly to 750.38 mg Trolox/100 mL, confirming the negative impact of elevated temperature on antioxidant retention.

Table 5. Antioxidant content determined by the FRAP assay in Mao juice stored at 4 °C and 30 °C over the storage period.

Sample of Mao juice	Storage temperature	Antioxidant content (mg Trolox / 100 mL)					
		Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
Sample A	4 °C	826.56	824.58	820.00	816.50	812.94	811.55
Sample B	4 °C	152.41	152.11	151.97	150.65	150.14	149.85
Sample C	4 °C	107.05	106.46	106.14	105.56	105.27	105.11
Sample D	4 °C	105.74	105.14	104.96	104.26	103.96	103.67
Sample A	30 °C	770.80	767.11	764.24	757.64	754.12	750.38
Sample B	30 °C	149.56	147.96	147.38	146.07	145.34	145.05
Sample C	30 °C	106.27	105.54	105.35	104.52	104.10	103.94
Sample D	30 °C	104.04	103.57	102.80	102.42	101.90	101.43

Table 6. Degradation rate constants and half-life ($t_{1/2}$) of antioxidants in Mao juice during storage at 4 °C and 30 °C, determined by the FRAP assay assuming first-order kinetics.

Sample of Mao juice	Storage temperature	Degradation rate constant $k \times 10^{-3}$ (day^{-1})	Half–life $t_{1/2}$ (week)
Sample A	4 °C	6.0	165.0
Sample B	4 °C	5.0	198.0
Sample C	4 °C	6.0	165.0
Sample D	4 °C	6.0	165.0
Sample A	30 °C	8.0	126.6
Sample B	30 °C	10.0	99.0
Sample C	30 °C	8.0	126.6
Sample D	30 °C	7.0	141.4

The degradation kinetics of antioxidants in Mao juice conformed to a first-order model, as illustrated in Figure 6 and summarized in Table 6. The rate constants (k) ranged from 5.0×10^{-3} to 10.0×10^{-3} day^{-1}, with the most rapid degradation observed in Sample B stored at 30 °C ($k = 10.0 \times 10^{-3}$ day^{-1}), corresponding to the shortest half-life of 99.0 weeks. In contrast, the slowest degradation was observed in the same sample kept at 4°C ($k = 5.0 \times 10^{-3}$ day^{-1}), exhibiting the longest half-life of 198.0 weeks. These findings suggest that the antioxidant compounds present in Mao juice, particularly in Sample B, may exhibit varying thermal stability, which differences could influence formulation, extraction efficiency, or matrix composition. Compared to the ABTS method, which also indicated a gradual loss of antioxidant capacity, the FRAP assay provided a more apparent distinction between storage temperatures in terms of degradation kinetics. While both assays revealed the stability advantage of refrigeration, the FRAP results particularly emphasized the susceptibility

of ferric–reducing antioxidants to heat–induced oxidation over time. This may imply differences in the types of antioxidants each method measures, with FRAP primarily detecting compounds capable of reducing Fe^{3+}, which are often more sensitive to thermal stress. The findings reinforce the necessity of cold storage to preserve the antioxidant integrity of Mao juice. Furthermore, combining insights from both FRAP and ABTS assays offers a more comprehensive understanding of antioxidant stability, as the two methods reflect different antioxidant mechanisms—electron transfer in FRAP versus radical scavenging in ABTS [38-42].

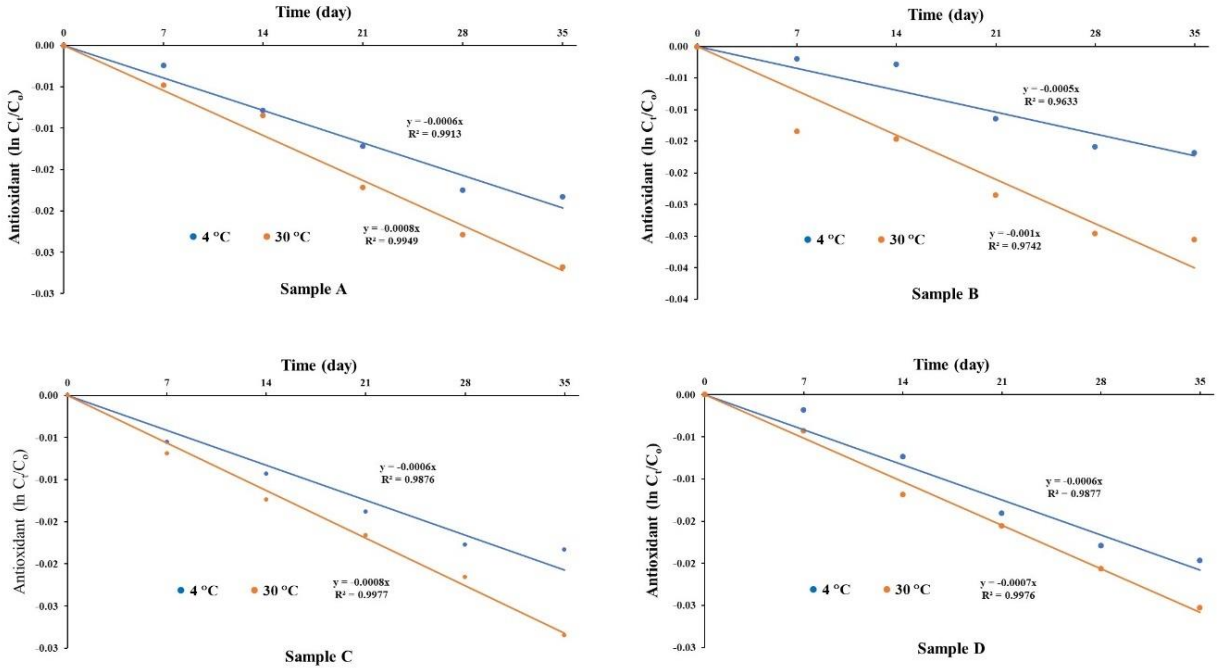

Figure 6. Degradation rate of antioxidants in Mao juice stored at 4 °C and 30 °C over 35 days, determined by the FRAP assay.

4. Conclusions

In summary, this study demonstrated that storage temperature and duration significantly affect the degradation of anthocyanins and the antioxidant activity in Mao juice, a community-produced beverage derived from *Antidesma thwaitesianum*. Among four commercial samples evaluated, Sample A stored at 4 °C exhibited the highest initial anthocyanin content (41.74 mg/L), which declined only slightly to 40.24 mg/L over 35 days. In contrast, at 30 °C, anthocyanin levels in the same sample decreased more rapidly, from 36.06 mg/L to 33.89 mg/L. Samples C and D, which had lower starting values (1.03 mg/L and 0.76 mg/L, respectively), exhibited marked degradation, dropping to 0.70 mg/L and 0.53 mg/L, respectively, at 30 °C. The degradation followed first–order kinetics, with rate constants ranging from 1.0×10^{-3} day^{-1} (Sample A at 4 °C) to 10.9×10^{-3} day^{-1} (Sample C at 30 °C), corresponding to half–lives between 99.0 and 9.1 weeks. Antioxidant activity, measured by the ABTS assay, confirmed these trends. Sample A exhibited the highest initial antioxidant level at 4 °C (1286.17 mg Trolox/100 mL), decreasing to 1240.16 mg after 35 days; however, at 30 °C, the decline was more pronounced, reaching 1184.46 mg. The FRAP assay supported this observation: Sample A decreased from 826.56 to 811.55 mg Trolox/100 mL at 4 °C and from 770.80 to 750.38 mg at 30 °C. The most rapid antioxidant degradation occurred in Sample B at 30 °C, with a rate constant of 10.0×10^{-3} day^{-1} and a half–life of 99.0 weeks (FRAP). These findings highlight the critical role of cold storage in preserving the nutritional and functional properties of Mao juice. Refrigeration at 4 °C not only slowed anthocyanin and antioxidant degradation but also extended product stability compared to storage at 30 °C. These insights are particularly relevant for local producers aiming to maintain product quality and extend shelf life. To further enhance stability and commercial viability, future development may focus on utilizing encapsulation technologies to protect bioactive compounds from oxidation and thermal stress. The addition of natural stabilizers or co-

pigments could help reduce degradation by enhancing the resilience of the pigment. Refinement of thermal or non-thermal processing methods, such as optimized pasteurization or high-pressure processing (HPP), may offer additional benefits. Moreover, selecting packaging with enhanced oxygen and light barrier properties can prevent premature degradation. Continued research into the bioavailability and health impacts of Mao juice under varying storage conditions will be essential for supporting its position as a functional beverage with regional and commercial significance.

5. Acknowledgements

This research was supported by the Chemistry Program and the Innovation in Chemistry for Community Research Unit at the Faculty of Science and Technology, as well as the Biomass Energy Research Laboratory within the Center of Excellence on Alternative Energy, Research and Development Institute at Sakon Nakhon Rajabhat University, Thailand. These institutions provided the necessary equipment and resources for the study.

Author Contributions: Conceptualization, T.S. and W.R.; methodology, T.S. and W.R.; validation, T.S. and W.R.; formal analysis, T.S., W.R., T.C., A.M.; investigation, T.C. and A.M.; resources, T.C. and A.M.; data curation, T.S. and W.R.; writing—original draft preparation, T.S. and W.R.; writing—review and editing, W.R.; visualization, T.S. and W.R.; funding acquisition, T.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Chemistry Program and the Innovation in Chemistry for Community Research Unit at the Faculty of Science and Technology, Sakon Nakhon Rajabhat University, Thailand.

Conflicts of Interest: The authors declare no conflict of interest.

Declarations: Declaration of generative AI and AI–assisted technologies in the writing process.

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