

Determining optimal storage conditions for Aloe-Buni functional drink: a stability assessment

[Menentukan kondisi penyimpanan optimal untuk minuman fungsional Aloe-Buni: penilaian stabilitas]

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ABSTRACT

Aloe-Buni functional drink contains vitamin C, anthocyanins, flavonoids, and antioxidant compounds that are highly sensitive to temperature and storage duration. The stability of these bioactive components determines the product's nutritional quality, health benefits, and overall shelf life. This study aimed to analyse the effects of temperature variation and storage duration to determine the optimal storage conditions and to estimate the functional shelf life of Aloe-Buni drink. The study was designed factorially using a completely randomized design, consisting of two factors: storage temperature (4°C, 13°C, and 27°C) and storage duration (0, 3, 6, 9, 12, and 15 days), and was conducted in two replications. The parameters analysed included pH, total flavonoids, total anthocyanins, vitamin C content, and antioxidant activity (radical inhibition), all measured by spectrophotometry. The data were analysed using ANOVA, followed by BNT to determine differences between treatments. All parameters were measured periodically to monitor the rate of bioactive degradation during storage. Storage temperatures of 4°C and 13°C were found to maintain better pH, vitamin C, anthocyanins, flavonoids, and antioxidant activity than that of 27°C, thereby slowing bioactive degradation during storage. Aloe-Buni drink have the best bioactive stability at low temperatures with a functional shelf life of approximately 6 day before significant deterioration occurs. This study provides a scientific basis for establishing storage standards and shelf-life for Aloe-Buni-based products and supports the development of stable, highly nutritious, locally produced functional drink.

Keywords: Aloe vera, Antidesma bunius, bioactive component stability, functional drink, storage conditions

ABSTRAK

Minuman fungsional Aloe-Buni mengandung vitamin C, antosianin, flavonoid, dan senyawa antioksidan yang sangat sensitif terhadap suhu dan lama penyimpanan. Stabilitas komponen bioaktif tersebut menentukan kualitas gizi, manfaat kesehatan, dan umur simpan produk. Penelitian ini bertujuan untuk mengevaluasi pengaruh suhu dan lama penyimpanan terhadap stabilitas minuman fungsional Aloe-Buni serta menentukan kondisi penyimpanan yang optimal dan perkiraan umur simpan fungsionalnya. Penelitian menggunakan Rancangan Acak Lengkap (RAL) dengan perlakuan suhu penyimpanan 4°C, 13°C, dan 27°C serta periode pengamatan 0, 3, 6, 9, 12, dan 15 hari. Parameter yang dianalisis meliputi pH, kadar vitamin C, total flavonoid, total antosianin, dan aktivitas antioksidan yang seluruhnya diukur menggunakan metode spektrofotometri. Data dianalisis menggunakan ANOVA yang dilanjutkan dengan uji BNT atau Duncan untuk mengetahui perbedaan antarperlakuan. Suhu penyimpanan sebesar 4°C dan 13°C terbukti lebih efektif dalam mempertahankan sifat fisikokimia dan komponen bioaktif dibandingkan suhu 27°C, sehingga mampu memperlambat degradasi senyawa bioaktif selama penyimpanan. Minuman fungsional Aloe-Buni menunjukkan stabilitas bioaktif terbaik pada penyimpanan suhu rendah dengan perkiraan umur simpan fungsional selama 6 hari sebelum terjadi penurunan mutu yang signifikan. Hasil penelitian ini memberikan dasar ilmiah untuk penetapan standar penyimpanan dan rekomendasi umur simpan produk berbasis Aloe-Buni serta mendukung pengembangan minuman fungsional lokal yang stabil dan bernilai gizi tinggi.

Kata kunci: Aloe vera; Antidesma bunius; stabilitas bioaktif; umur simpan; suhu penyimpanan

Introduction

Aloe-Buni was a functional drink formulated from Aloe Vera gel and Buni fruit (Suriati et al., 2025). These two natural ingredients are rich in bioactive compounds, including vitamin C, flavonoids, and anthocyanins. These compounds play an essential role in supporting health through their antioxidant activity, which can neutralise free radicals. Aloe Vera gel was rich in phenolic compounds, flavonoids, and polysaccharides, which contribute to its antioxidant properties (Cuvás-Limón et al., 2022). Aloe Vera gel offered many health benefits, such as anti-inflammatory (Catalano et al., 2024; Javed & Zafar, 2025), antioxidant (Raluca et al., 2022), antimicrobial (Dena et al., 2023), antifungal (Salim et al., 2024), and antidiabetic properties (Prakash et al., 2023). Aloe Vera gel also aided in wound healing, lowers blood sugar levels, improves lipid profiles, and boosts immune function (Moselhy et al., 2024). In addition to aloe vera, this drink also contains Buni fruit extract. The addition of Buni fruit to the Aloe Vera gel enhanced the taste, visual appeal, and antioxidant capacity of the drink (Suriati et al., 2025). The bioactive combination of these two ingredients makes Aloe-Buni a potential value-added functional drink. This combination drink optimised the antioxidant properties of Aloe Vera and Buni, thereby potentially boosting the immune response and reducing oxidative stress, and significantly improving the regulation of metabolic disorders (Sharma et al., 2024). However, optimal utilisation of this potential was greatly influenced by the stability of bioactive components during storage (Gupta et al., 2023).

The stability of bioactive components in functional drinks is a crucial factor in ensuring the product's physiological effectiveness. Compounds such as vitamin C and anthocyanins were able to degrade progressively due to oxidation, photodegradation, and enzymatic activity (Alabbosh et al., 2025; Lema et al., 2022). This decrease in stability not only affects the product's nutritional quality but also undermines its functional claims. The degradation of vitamin C, flavonoids, and anthocyanins reduced antioxidant activity, rendering the drink's functional claims no longer reflective of its physiological effectiveness (Huang et al., 2022). These changes are often accompanied by a decline in sensory quality, including colour fading due to anthocyanin degradation, changes in taste, and the development of an unpleasant aroma. Chemically and physically, the decline in pH and the progression of oxidative reactions during storage might destabilize the gel matrix, thereby affecting the viscosity, clarity, and overall quality of the product (Zhang et al., 2023). Therefore, a thorough understanding of the factors that affect bioactive stability is essential to ensure the quality of Aloe-Buni drink throughout its shelf life.

Storage temperature significantly affects the rates of chemical and microbiological reactions in food products. Low temperatures slow chemical degradation and microbial growth, thereby extending shelf life and preserving bioactive compounds in functional drinks (Kumar et al., 2024). Kumar et al. (2024) reported that kiwi juice had a longer shelf life at 5°C due to reduced microbial growth and bioactive degradation, while Muniandy et al. (2022) found that vitamin C degradation occurred more slowly at 4°C than at higher temperatures. These findings confirm the importance of temperature control in preserving heat-sensitive functional components. However, comprehensive information on the stability of Aloe-Buni bioactive compounds under different storage temperatures and durations remains limited. The optimal temperature and shelf life for preserving vitamin C, flavonoids, anthocyanins, and antioxidant activity have not yet been established, making accurate shelf-life labelling difficult. Therefore, this study aimed to determine the optimal storage temperature and duration for Aloe-Buni drink by evaluating changes in physicochemical properties and bioactive compounds, as well as estimating its shelf life under different storage conditions. The drink was stored at 4°C (refrigerated), 13°C (cool), and 27°C (ambient tropical conditions) for up to 15 days. Changes in pH, vitamin C, total flavonoids, total anthocyanins, and antioxidant activity were evaluated. The results are expected to provide optimal storage recommendations for industries and Micro, Small, and Medium Enterprises (MSMEs) to maintain the quality and functional benefits of Aloe-Buni drink.

Materials and methods

Materials and equipment

The main ingredient used is Aloe Vera leaves (*Aloe vera Barbadensis* M.) from Denpasar, Bali, Indonesia, that are more than 1 year old. One-year-old Aloe Vera leaves do not have white spots and weigh 500g–1 kg per leaf. Buni fruit (*Antidesma bunius* L.) from Tabanan, Bali, Indonesia is 2.5 to 3 months old after flowering. The buni fruit is red. The materials used in the analysis during the study included filter paper, alcohol (Smart Lab), distilled water, 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma Aldrich), methanol (Smart Lab), ascorbic acid (Merck), sulfuric acid (Merck), sodium phosphate (Merck), ammonium molybdate (Merck), 10% AlCl_3 (Merck), 10% NaNO_2 (Merck), 6 N HCl (Merck), and 0.5% HCl (Merck). The equipment used for analysis included dropper pipettes (Pyrex), volumetric pipettes (Pyrex), beakers (Pyrex), measuring cups (Pyrex), erlenmeyer flasks (Pyrex), jars, test tubes (Pyrex), funnels (Pyrex), analytical balances (Ohaus, USA), pH meter (Hanna HI 8424, Europe), magnetic stirrer (VWR IKA VMS-C7, USA), UV-Vis spectrophotometer (Libra S60, USA), and shaker (Ohaus, USA).

Research methods

This study used a completely randomised design (CRD) with two factors. The first factor was storage temperature, namely 4°C, 13°C, and 27°C. The second factor was storage days, namely days 0, 3, 6, 9, 12, and 15 (Suriati et al., 2025). Each treatment was repeated twice. The results obtained from this study were analysed using ANOVA fingerprints. Results from various treatment fingerprints that had a significant effect ($P < 0.05$) to a very significant effect ($P < 0.01$) were followed up with a 5% BNT test.

Research procedures

The Aloe–Buni drink was prepared by processing Aloe vera gel (aloe vera) and Buni fruit juice (*Antidesma bunius*). Fresh Aloe Vera leaves were peeled to separate the rind and latex layer. The Aloe Vera flesh was washed under running water to reduce mucilage, then ground to obtain a slurry. The Aloe Vera slurry was pasteurized at 80 °C for 15 minutes to reduce microbial load and inactivate deteriorative enzymes that may accelerate quality degradation. Ripe Buni fruits were sorted and washed, then crushed using a blender and filtered to separate seeds and pulp in order to obtain the juice. The Buni juice was subsequently pasteurized at 80 °C for 15 minutes to inhibit microbial growth and maintain the stability of bioactive compounds. The Aloe Vera slurry and Buni juice were mixed according to the predetermined formulation and homogenized until uniform. The mixture was then cooled to room temperature and filled into sanitized plastic bottles, which were tightly sealed to prevent contamination. After packaging, the products were stored at different storage temperatures. Quality analyses were conducted on storage days of 0, 3, 6, 9, 12, and 15.

Research parameters

The parameters analysed included pH adapted from the method of Suriati et al. (2025), vitamin C content adapted from the technique Vuong et al. (2014), total flavonoids adapted from the method Singh et al. (2012), total anthocyanin adapted from the technique Unes et al. (2005), and antioxidant activity adapted from the method Salim et al. (2017).

Results and discussion

1) pH

The results of the analysis of variance showed that storage-temperature and storage time-significantly affected on the pH of the Aloe-Buni drink, however, there was no interaction between storage temperature and storage time on the pH of the Aloe-Buni drink. Before the storage treatments were applied, the initial pH of the Aloe-Buni drink was 3.30. The pH values of Aloe-Buni functional drink showed significant differences across storage temperature treatments. Storage at 13°C showed the highest pH level (± 3.30),

followed closely by storage at 4°C (± 3.28). In contrast, the lowest pH level was recorded at 27°C (± 3.05) (see Figure 1). The relatively small but significant difference in pH between 4°C and 13°C indicates that both low temperatures effectively slowed the formation of acidic degradation products during storage. Lower temperatures reduce the oxidation of ascorbic acid and other oxidative reactions, thereby limiting the accumulation of organic acids that contribute to pH reduction. The slightly more stable pH observed at 13°C suggests that this temperature maintained the physicochemical stability of the Aloe-Buni drink while minimizing deterioration of its matrix. In contrast, although storage at 4°C substantially slowed degradation reactions, oxidative processes could still occur at a reduced rate. These findings confirm that storage temperature plays a crucial role in maintaining the pH stability of the Aloe-Buni drink. Conversely, a lower temperature of 4°C can slow the reaction but does not entirely stop the natural oxidative degradation (Kumar et al., 2024; Muniandy et al., 2022). This pattern confirms that temperature is a very decisive factor in pH stability during storage. Lower temperatures can maintain a high pH because the rate of chemical degradation reactions is slower than at room temperature. (Wu et al., 2022). Physicochemically, a decrease in pH at 27°C indicates the development of various reactions that produce acidic compounds. At high temperatures, vitamin C oxidation occurs more rapidly, producing dehydroascorbate and organic acid derivatives that increase the system's acidity (Peleg, 2019). Flavonoids and anthocyanins are also more susceptible to hydrolysis and oxidation at high temperatures, producing more acidic phenolic fragments. This is in line with research by Yan et al. (2020), which found that high temperatures damage flavonoids, altering their structure and leading to the formation of acidic compounds. Flavonoids and anthocyanins are stable in acidic environments, but acidic conditions cannot prevent degradation if these compounds are stored at high temperatures (Pan et al., 2021; Polat Kaya et al., 2024).

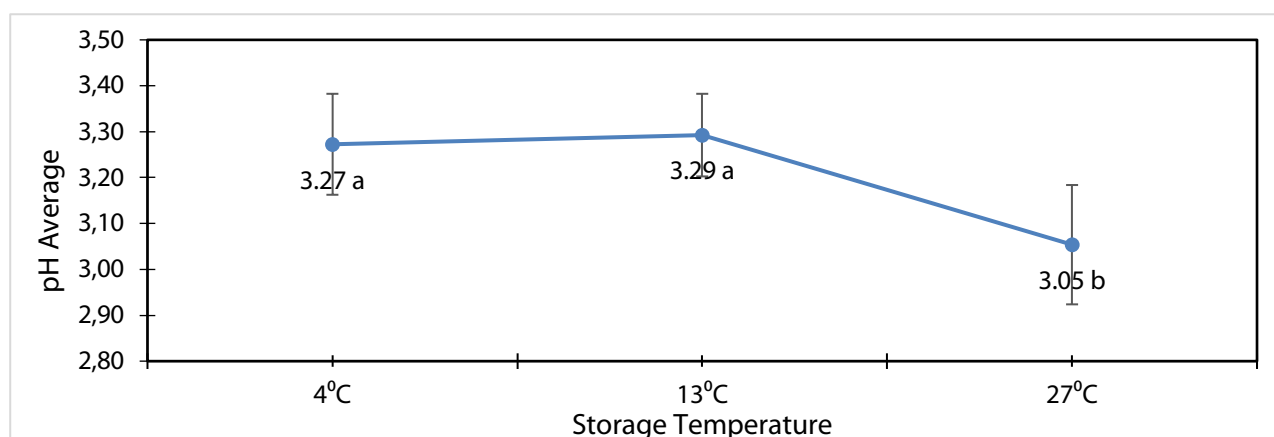


Figure 1. Graph of the pH value of Aloe-Buni functional drink at different temperatures

The pH value of Aloe-Buni functional drink showed a gradual downward trend during 15 days of Storage (Figure 2). On days 0 and 3, the pH was in the same range, around 3.30, indicating that conditions remained stable in the early stages of storage. Starting on day 9, the pH decreased from around 3.26 to around 3.03 on day 15. Although this decrease appears small numerically, the change is chemically significant because it reflects the accumulation of acidic degradation products in the drink system. Even though the decrease in pH was numerically small, it indicated the progressive oxidation of ascorbic acid into dehydroascorbic acid, followed by the formation of 2,3-diketogulonic acid and other acidic degradation products during storage (Yin et al., 2022). The decrease in pH during storage is primarily caused by the degradation of bioactive compounds, which produces various acidic degradation products. Additionally, changes in the Aloe Vera polysaccharide gel matrix affected molecular interactions and the physicochemical stability of the drink system, which in turn was able to influence the stability and degradation rate of bioactive compounds and indirectly contributed to changes in pH during storage (Rahman et al., 2025). Vitamin C (ascorbic acid) underwent oxidation to form dehydroascorbate, which subsequently broke down into

various organic acids, increasing the concentration of H^+ ions and decreasing the pH. These degradation processes collectively contributed to the gradual decline in pH observed during storage (Fenger et al., 2020; Rodriguez-Amaya & Carle, 2020).

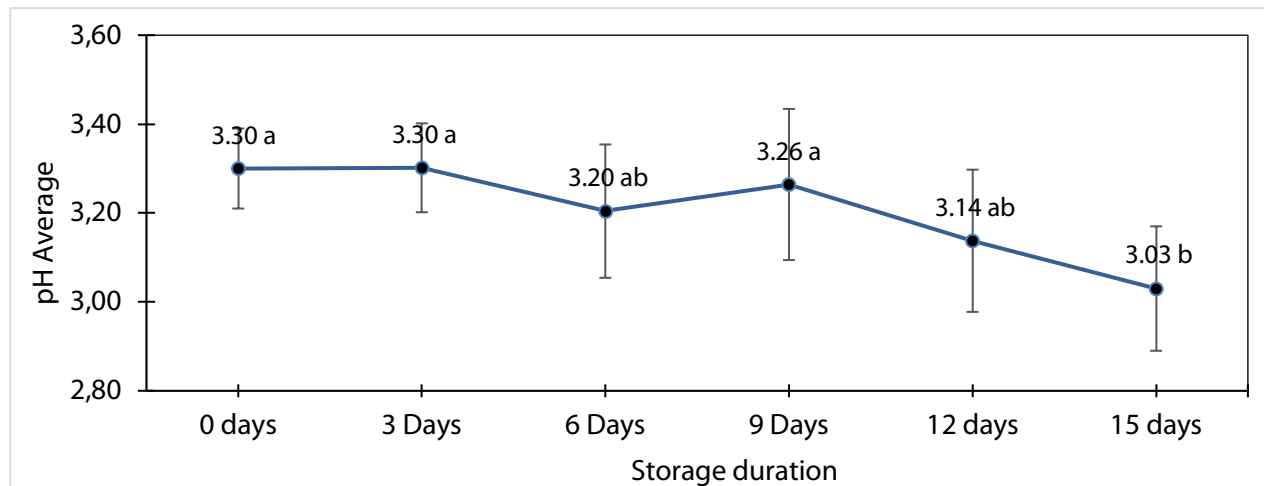


Figure 2. Graph showing the pH value of Aloe-Buni functional drink over the storage duration

2) Vitamin C

The results of the analysis of variance showed that temperature treatment had no significant effect on vitamin C content in the Aloe-Buni functional drink., however, storage time had a highly significant effect on vitamin C content in the Aloe-Buni functional drink. There was no interaction between storage temperature and storage time on the vitamin C content in the Aloe-Buni drink. The vitamin C content of the Aloe-Buni functional drink decreased steadily with increasing storage time. On day 0, the vitamin C content was at its highest level, around 160–180 mg AAE/100 g, then gradually decreased until day 15. The sharpest decline occurred in the early storage phase, from day 3 to day 6. After day 6, the decrease in vitamin C content continued until day 15, but at a low rate (Figure 3).

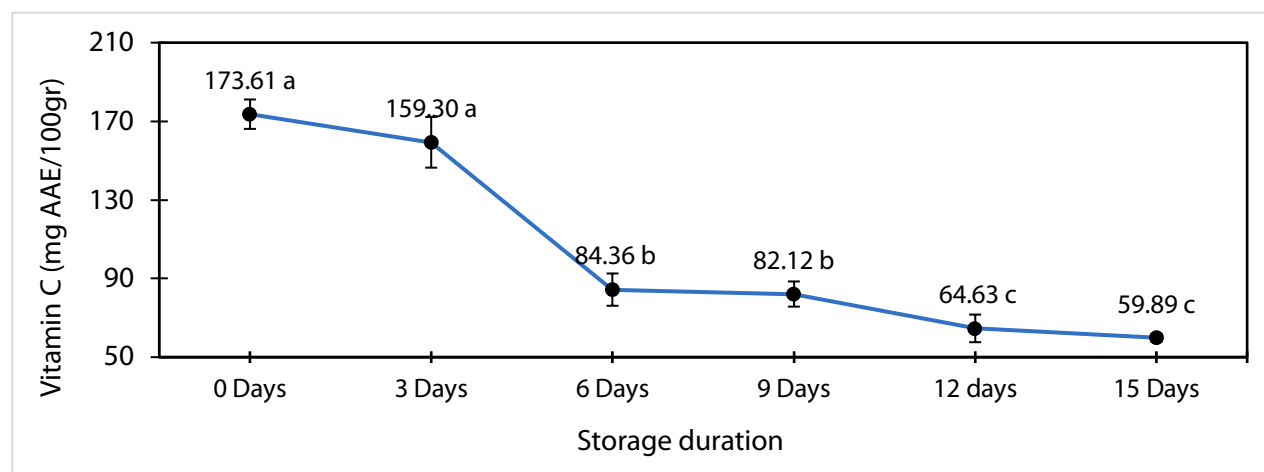


Figure 3. Graph showing the concentration of vitamin C in Aloe-Buni functional drink over the storage duration

This pattern indicates that the vitamin C content in the Aloe-Buni drink is highly susceptible to degradation from the start of storage, and its stability continues to decline throughout the storage period. This is in line with the results of the study by Zhang et al. (2020), which found that Vitamin C content in strawberry juice degraded at an increasing rate as it was stored longer. This downward trend is consistent with the nature of vitamin C as an oxidation-sensitive compound that is readily oxidised, especially in liquid systems such as drinks. During Storage, ascorbic acid underwent oxidation due to enzyme activity, converting to dehydroascorbic acid and subsequently decomposing into various organic acids, thereby

reducing its concentration in the product (Feng et al., 2020). This aligns with research by Anisimova (2022), which found that temperature and duration of storage affected the amount of ascorbic acid content in berries. The rate of degradation was higher in the early days due to the high vitamin C concentration and the availability of dissolved oxygen in the drink, leading to more intense oxidation reactions (Nakilcioğlu-Taş & Ötleş, 2020). As vitamin C content decrease, the rate of degradation slows down, resulting in a flatter decline in the graph during the final days of Storage. These findings suggested that both storage duration and storage temperature play important roles in determining vitamin C stability, and that low-temperature storage could help preserve the nutritional quality of Aloe-Buni functional drink during storage (Nakilcioğlu-Taş & Ötleş, 2020)

3) Total anthocyanins

The analysis of variance results indicated that storage temperature, storage time treatments, and the interaction of both treatments had no significant effect on total anthocyanins of the Aloe-Buni functional drink. The total anthocyanin content of Aloe-Buni functional drink at various temperatures and storage periods showed relatively small and fluctuating changes. The initial anthocyanin value on day 0 was 73 mg/100 g across all storage temperatures. The value remained within a relatively narrow range for each temperature treatment during a storage period of up to 15 days. Statistical analysis shows that neither storage temperature nor storage duration had a significant effect on total anthocyanin content ($p>0.05$), as reflected in the average daily anthocyanin values marked with the same letter throughout the storage period.

Table 1. Total anthocyanins (mg/100 g) in Aloe-Buni functional drink at different temperature and storage times

Temperature (°C)	Storage duration (days)					
	0	3	6	9	12	15
4	73.29±2.84	76.87±13.06	60.56±0.05	60.48±12.64	60.56±0.05	55.12±14.96
13	73.22±1.63	80.29±3.69	80.22±14.17	67.63±3.48	59.74±1.37	73.29±6.43
27	72.92±0.05	56.76±14.22	67.33±1.69	64.58±2.58	72.25±10.74	61.75±2.48

Anthocyanins are generally more stable in acidic conditions, and Aloe-Buni drink had a low pH (approximately 3.0–3.3) to support the presence of anthocyanins in the form of flavylum cations, which were more resistant to degradation (Hardinasinta et al., 2022). In addition, the presence of other phenolic compounds and Aloe Vera gel polysaccharides had the potential to form interactions that stabilised the anthocyanin structure, thereby reducing the rate of decomposition despite temperature variations (Lema et al., 2022). Unlike vitamin C, which is highly sensitive, the anthocyanin fraction in Aloe-Buni is relatively resistant to the storage conditions tested. Although storage at 13°C exhibited the highest anthocyanin retention after 15 days, the differences among temperature treatments were not statistically significant ($p>0.05$). Therefore, anthocyanins may still contribute to the antioxidant activity of Aloe-Buni drink throughout the storage period evaluated in this study.

4) Total flavonoid

The results of the analysis of variance showed that temperature treatment had no significant effect on total flavonoid content in the Aloe-Buni functional drink, whereas storage time had a highly significant effect on total flavonoid content in the Aloe-Buni functional drink. There was no interaction between storage temperature and storage time on the total flavonoid content in the Aloe-Buni drink. The total flavonoid content in Aloe-Buni functional drink showed a consistent downward trend over the 15-day storage period. On day 0, the flavonoid content was at its highest, around 300 mg QE/100 g. Flavonoids declined sharply until day 3. This decline continued on the sixth day, with values still lower than on previous days, indicating that the initial storage phase was the most critical period for flavonoid degradation. From

the ninth to the fifteenth day, the rate of flavonoid decline appeared to be more stable and less drastic than in the early days, although flavonoid values continued to decline gradually (Figure 4).

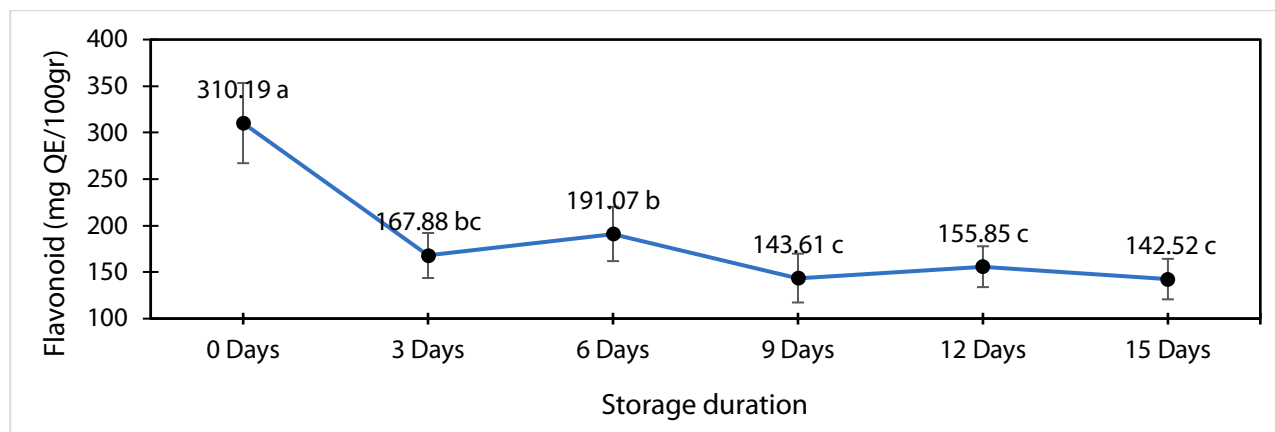


Figure 4. Graph of total flavonoids in Aloe-Buni functional drink over storage duration

Flavonoids are sensitive to oxidation, temperature, oxygen, light, and pH changes. Oxidative degradation occurs more rapidly during early storage, producing quinones and other phenolic compounds that reduce flavonoid content and antioxidant capacity (Liu et al., 2020; Shubina et al., 2023). Decreasing pH may further destabilize flavanols and flavanones through structural changes (Oganessian & Shatokhin, 2021; Picos-Salas et al., 2025). The interaction of hydrogen and the complexation between flavonoids and polysaccharides provided a partial protective effect, thereby slowing further degradation (He et al., 2025; Wang et al., 2025). The slower decline after day 9 suggests that reactive fractions had mostly degraded earlier, while remaining flavonoids were relatively protected by Aloe vera polysaccharides through complexation, which can improve their stability and slow further degradation (Cao et al., 2020; He et al., 2025; Wang et al., 2025).

5) Antioxidant activity (radical inhibition)

The results of the analysis of variance showed that storage temperature treatment had no significant effect on antioxidant activity (radical inhibition) of the Aloe-Buni functional drink, but storage time treatment had a highly significant effect on antioxidant activity (radical inhibition) of the Aloe-Buni functional drink. There was no interaction between storage temperature and storage time on antioxidant activity (radical inhibition) of the Aloe-Buni functional drink. The antioxidant activity of the Aloe-Buni functional drink showed a consistent downward trend during the 15-day storage period. On day 0, antioxidant activity reached its highest level of approximately 33 percent, reflecting the initial contribution of vitamin C, flavonoids, and anthocyanins as free radical scavengers. On day 3, antioxidant activity levels remained relatively high and did not differ significantly from day 0, indicating that the antioxidant mechanism remained optimally active in the early stages of storage. However, from day 6 to day 15, the graph showed a more pronounced and steady decline, with antioxidant activity falling to around 19–22 percent inhibition at the end of storage (Figure 5).

The decrease in antioxidant activity was mainly attributed to the oxidative degradation of vitamin C and flavonoids during storage. Higher temperatures accelerated the oxidation of vitamin C and flavonoids, reducing their ability to neutralize free radicals (Yin et al., 2022; Liu et al., 2020). Prolonged storage further increased cumulative degradation, resulting in a gradual decline in radical-scavenging activity (Chouikh, 2025). Although anthocyanins were relatively more stable, their contribution to total antioxidant capacity was lower; thus, their stability helped prevent a sharp decline in antioxidant activity. This was reflected in the gradual decrease shown in Figure 5, which may also be supported by interactions between anthocyanins and Aloe vera polysaccharides or other phenolic compounds (Chandra et al., 2024; Zhang et al., 2025).

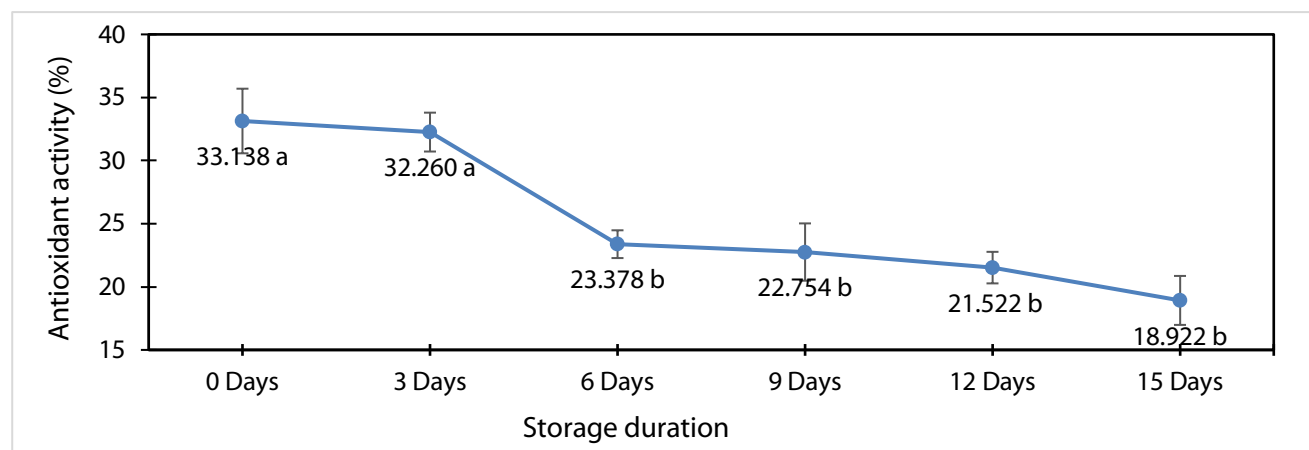


Figure 5. Graph of the antioxidant activity of Aloe-Buni functional drink against storage duration

Conclusion

The results showed that storage temperature and storage duration affected the physicochemical and bioactive stability of Aloe-Buni functional drink. Overall, low-temperature storage (4°C and 13°C) was more effective in maintaining pH, vitamin C, flavonoids, and antioxidant activity than storage at room temperature. A significant decrease in vitamin C levels, flavonoid content, and antioxidant activity was observed after 6–9 days of storage, indicating that product quality may begin to decline after that period. These findings provide a scientific basis for determining storage conditions and serve as preliminary information for future real-time stability studies aimed at determining the shelf life of Aloe-Buni functional drink.

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