



Effect of Storage Conditions on the Phenolic Content and Antioxidant Properties of Freeze-Dried *Bignay* [*Antidesma bunius* (L.) Spreng.] Pomace Extract

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Abstract— *Bignay* pomace, a fruit wine byproduct, is rich in phenolics with significant potential for valorization into a functional food ingredient. However, it is prone to degradation during storage. This study investigated the effects of temperature (−20°C, 4°C, 30°C, and 40°C) and lighting conditions on the stability of phenolic compounds and antioxidant properties of freeze-dried *bignay* pomace obtained through an eco-friendly, water-based extraction method. Total phenolic content (TPC), DPPH radical scavenging activity, and ferric reducing antioxidant power (FRAP) were monitored over a 35-day period. Across all time points, samples at −20°C and 4°C generally retained higher TPC and antioxidant activity than those stored at 30°C and 40°C. Notable fluctuations were observed, with an early decline in the first weeks, a brief rise around Days 21–28, and a final drop by Day 35. After 35 days, TPC declined by ~18–21% at −20°C, 4°C, and 30°C, while at 40°C the reduction reached ~25%. Degradation followed zero-order kinetics, with rate constants (k) ranging from 3.2224 to 4.5207, and the slowest degradation at −20°C. DPPH activity decreased by 23–33% and FRAP by 41–64%, with the most significant losses at 40°C. Light had a moderate effect, significantly influencing DPPH scavenging activity at 4°C and FRAP at −20°C and 30°C only. Overall, temperature was the primary factor influencing degradation. Keeping the extract at low temperatures (freezing or refrigeration) is critical to preserving its phenolic content and antioxidant properties for use as a natural, clean-label ingredient, while light protection plays a secondary role.

Keywords— freeze-drying; *bignay* pomace; antioxidant activity; storage conditions; total phenolic content; degradation kinetics

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I. INTRODUCTION

Antidesma bunius, commonly known as *bignay*, is a wild fruit plant naturally found in the lower Himalayas in India, Sri Lanka, and Southeast Asia, including the Philippines, Thailand, Indonesia, and Malaysia, as well as in Papua New Guinea, the Solomon Islands, and northern Australia [1]. It is referred to by various names, such as *bignay* in the Philippines, *berunai* in Malaysia, *mao luang* in Thailand, *kho line tu* in Laos, *hooni* in Indonesia, *choi moi* in Vietnam, and *moikin* and *chunka* in

Australia [2]. Thriving in tropical and subtropical climates, it is highly adaptable to a wide range of soil conditions [3].

Bignay fruits grow in clusters, with round to slightly ovoid berries that ripen in a mix of green, yellow, red, purple, and nearly black hues within a single bunch. Each fruit contains a single seed, and when fully ripe, the pulp becomes juicy and purplish to black. The fruit is moisture-rich, with levels ranging between 64.47% and 88% [3–4]. On a dry basis, it is primarily composed of carbohydrates (~90%) and crude fiber (~9.43%), while its protein and crude fat account for only 1.23% and

0.97%, respectively [4]. The edible portion has a moisture content of 90–95% and contains 6.3% carbohydrates, 0.8% fat, 0.7–0.75% protein, and 0.57–0.78% ash [1,5]. Additionally, it is a good source of vitamins, minerals, and various organic acids [2-3], [5-6].

Bignay has gained increasing attention due to its antioxidant properties and high phenolic content. Studies have identified several phenolic compounds, including flavonoids, saponins, tannins, phenolic acids, terpenes, pro-cyanidin B1 and B2, and anthocyanins [5]. The major flavonoid components are (-)-epicatechin and (+)-catechin (flavan-3-ols), while the dominant anthocyanins are cyanidin, malvidin, pelargonidin, and delphinidin [7-9]. The primary phenolic acids found in the fruit are gallic acid, ellagic acid, and vanillic acid, along with five other hydroxycinnamic acids—caffeic acid, p-coumaric acid, ferulic acid, sinapinic acid, and cinnamic acid [7-8]. Studies on *bignay* grown in the Philippines revealed that the pulp had total phenolic content (TPC) ranging from 1839 to 1978.38 mg gallic acid equivalent/100 g DW, while the total anthocyanin content was 131.42 mg cyanidin-3-glucoside equivalent (C-3GE)/100 g DW [10-11]. These findings collectively demonstrate that *bignay* is a phenolic-rich fruit with considerable antioxidant potential.

Bignay is an excellent material for making jams, jellies, preserves, juice or juice concentrate, and wine. In the Philippines, the fruit wine industry has been growing, with *bignay* being a preferred substrate for winemaking. However, only the puree is used in processing, leaving a significant amount of byproduct. Concerns about food processing waste and environmental sustainability have led to an increased interest in valorizing fruit-processing byproducts, aligning with the principles of the circular economy.

Bignay pomace, composed of seeds, pulp, and skin, exhibits strong functional potential due to its bioactive composition. It contains substantial amounts of phenolic compounds and dietary fiber. Because *bignay* pomace retains small portions of the pulp, it likely contains minor quantities of minerals and organic acids. A study by Zubia et al. [12] confirmed significant levels of phenolic compounds in fresh and dried *bignay* pomace (convection oven-dried and freeze-dried). The extraction of bioactive compounds from fruit pomace presents opportunities for various applications, including the food industry. It holds great commercial potential, consistent with the growing use of phenolic-rich extracts in active food packaging and edible coatings. Phenolic compounds are effective natural antioxidants and antimicrobial agents that help prevent lipid oxidation and microbial spoilage, thereby extending the shelf life of products [13]. For instance, grape pomace extract has been successfully applied in meat products to enhance acceptability and shelf life [14]. Food manufacturers can use *bignay* pomace extract as a natural preservative and bioactive ingredient for similar applications.

A sustainable, solvent-free extraction method for recovering phenolic compounds from *bignay* pomace has been optimized

by Babaran et al. [15]. The eco-friendly process uses only distilled water, ensuring that the extract is free from chemical solvents. As a result, the *bignay* pomace extract can be directly incorporated into food products without the need for further purification and processing, making it an attractive ingredient for clean-label and natural formulations. Additionally, the method is cost-friendly, as it eliminates the need for expensive solvents and complex processing steps, offering an economical alternative for producing high-quality extracts. Despite these developments in extraction methods, information on how solvent-free *bignay* pomace extracts behave during storage is still lacking.

However, phenolic compounds are sensitive to degradation due to environmental factors such as light and temperature variations [16]. Recent findings highlight storage temperature as a crucial factor in preserving phenolic compound extracts from plant-based matrices. Gómez-Mejía et al. [17] reported that extracts from lemon and clementine peels could remain stable at 20°C. At the same time, Thitilertdech [18] found that *Nephelium lappaceum* rind extract maintained up to 88.8% of its phenolic content and antioxidant potential after 16 weeks at 4°C. These findings demonstrate that lower storage temperatures can effectively minimize temperature-induced degradation of phenolics and their associated antioxidant functions. However, the stability and degradation kinetics of water-extracted phenolics from *bignay* pomace under different storage temperatures and lighting conditions remain unexplored. Understanding these factors is crucial to ensure the preservation of their beneficial properties and biological activities.

Therefore, this study aimed to assess the effects of temperature and lighting conditions on the stability of solvent-free, water-extracted phenolic compounds from *bignay* pomace. To the best of our knowledge, this is the first study to evaluate its storage stability and degradation kinetics. The findings will provide valuable insights into optimizing storage conditions to maintain the integrity and functionality of its bioactive compounds. By generating quantitative information on phenolic retention and antioxidant activity, this work supports the potential of *bignay* pomace extract as a natural, clean-label ingredient in various food applications.

II. MATERIAL AND METHODS

A. Materials

Analytical-grade chemicals and reagents were used in the analyses of samples. Folin-Ciocalteu, sodium carbonate, 2,2-diphenyl-2-picrylhydrazyl, 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride, methanol, gallic acid (3,4,5-trihydroxybenzoic acid), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma-Aldrich Co. (Singapore).

Fully ripe *bignay* fruits (deep purple to black) were harvested from trees within the vicinity of the University of the Philippines Los Baños, Laguna. The fruits were processed

using a pulper finisher (Kiya Seisakusho, Japan) to obtain the pomace. The pomace was stored in the freezer and then freeze-dried at 25–30°C heater temperature and -30°C chilling temperature until the final moisture content reached 6%, which was achieved after 30 hours. The freeze-dried pomace was vacuum-packed in 20 g portions and stored in the freezer at -20°C until use.

B. Methods

Extraction

Thawed freeze-dried *bignay* pomace was ground and passed through a 20-mesh sieve. Extraction of phenolics from the homogenized powder was carried out based on the optimized conditions established by Babaran et al. [15]. The extraction process was performed using a solute-to-solvent ratio of 1.04:10 (pomace/distilled water, w/v). The distilled water used was at pH 6.6. The mixture was then placed inside the incubator (Biobase BOV D50) at 30°C for 13 min. The supernatant was filtered through Whatman® No. 1 filter paper (Sigma-Aldrich, U.S.A.), and the filtrate (extract) was collected.

Storage design and sampling procedure

The *bignay* pomace extract used for the storage study was prepared from a single batch of homogenized extract to ensure uniformity across treatments. Five mL of the extract was filled in transparent glass vials for samples with light exposure, and for samples without light, the extract was transferred to vials wrapped in aluminum foil. Vials were maintained at -20, 4, 30, and 40°C for 35 days. For each treatment, 18 vials were prepared. The samples were withdrawn after 1, 7, 14, 21, 28, and 35 days. Three representative vials per treatment were randomly selected for analysis. The stability of the bioactive compounds was monitored by determining total phenolic content and antioxidant activity (DPPH and FRAP methods).

Quantification of total phenolics

The total phenolic content was analyzed using the Folin-Ciocalteu method based on ISO (International Organization for Standardization) 14502-1 with minor modifications [19]. Appropriately diluted *bignay* pomace extract (0.30 mL) was mixed with 1.5 mL of 10% (v/v) Folin-Ciocalteu reagent, followed by the addition of 1.2 mL of 7.5% (w/v) sodium carbonate. The mixture was vortexed and left in the dark at room temperature for 1 hour. Using a double-beam UV-vis spectrophotometer (Shimadzu Corp., Japan), the absorbance was measured at 765 nm against a blank. Gallic acid was used as standard. TPC was calculated and expressed as µg gallic acid equivalent per mL (µg GAE/mL) of extract.

Antioxidant Assays

DPPH radical scavenging activity

The antioxidant activity of *bignay* pomace extract was assessed following a standard assay for 2,2-diphenyl-2-picrylhydrazyl based on the method of Zubia et al. [12]. The reaction mixture was prepared by mixing 1.5 mL of diluted extract with 1.5 mL of freshly prepared 60 µM methanolic solution of the free radical DPPH. The solution was mixed using a vortex mixer and

kept in the dark at room temperature for 30 min. A calibration curve was generated using different concentrations of Trolox standard, and the absorbance was measured at 517 nm using absolute methanol as a blank. The results were expressed as µg Trolox equivalent (TE) per mL (µg TE/mL) extract. All analyses were performed in four replicates.

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power assay was performed according to a standard method described by Tomasina et al. [20], and all analyses were done in triplicate. The FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM ferric chloride in a 10:1:1 (v/v/v) ratio. The solution was incubated for 30 min while maintaining a temperature of 37°C. Thereafter, 2.7 mL of FRAP reagent was mixed with 0.3 mL of appropriately diluted sample and incubated further at 37°C for 5 min. All solutions were freshly prepared on the day of the analysis. The absorbance was measured at 620 nm, and the results were expressed as µg Trolox equivalent (TE) per mL (µg TE/mL) of extract.

Kinetic modelling of total phenolics

The degradation of phenolic compounds at different temperatures and lighting conditions was evaluated by fitting the experimental data to zero-, first-, and second-order kinetic models. The calculation was obtained from the linear regression equation from plotting the concentrations of phenolics against storage time (Equation 1).

$$Y = a + kx$$

where Y is the total phenolic content, x is the storage time (day), and k is the rate constant.

The kinetic models were determined through the following equations (2–4):

$$\text{Zero-order: } C - C_0 = -kt$$

$$\text{First-order: } \ln \frac{C}{C_0} = -kt$$

$$\text{Second-order: } \frac{1}{C} - \frac{1}{C_0} = kt$$

where C is the concentration of total phenolics in *bignay* pomace at a specific time, C₀ is the initial concentration, k is the reaction rate constant, and t is time in days.

Statistical analysis

All measurements were performed in three replicates, unless otherwise stated. Results were expressed as mean ± standard deviation. Data were tested for homogeneity of variance using Levene's test. One-way analysis of variance (ANOVA) ($\alpha = 0.05$) was then applied to compare treatments. Tukey's Honestly Significant Difference (HSD) test was used to determine significant differences among means at a 95% level of confidence. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp, New York, U.S.A.).

III. RESULT AND DISCUSSION

A. Total phenolic content and degradation kinetics of bignay pomace extract

Changes in the total phenolic content over time

The total phenolic content (TPC) of freeze-dried bignay pomace extract was monitored for 35 days under different

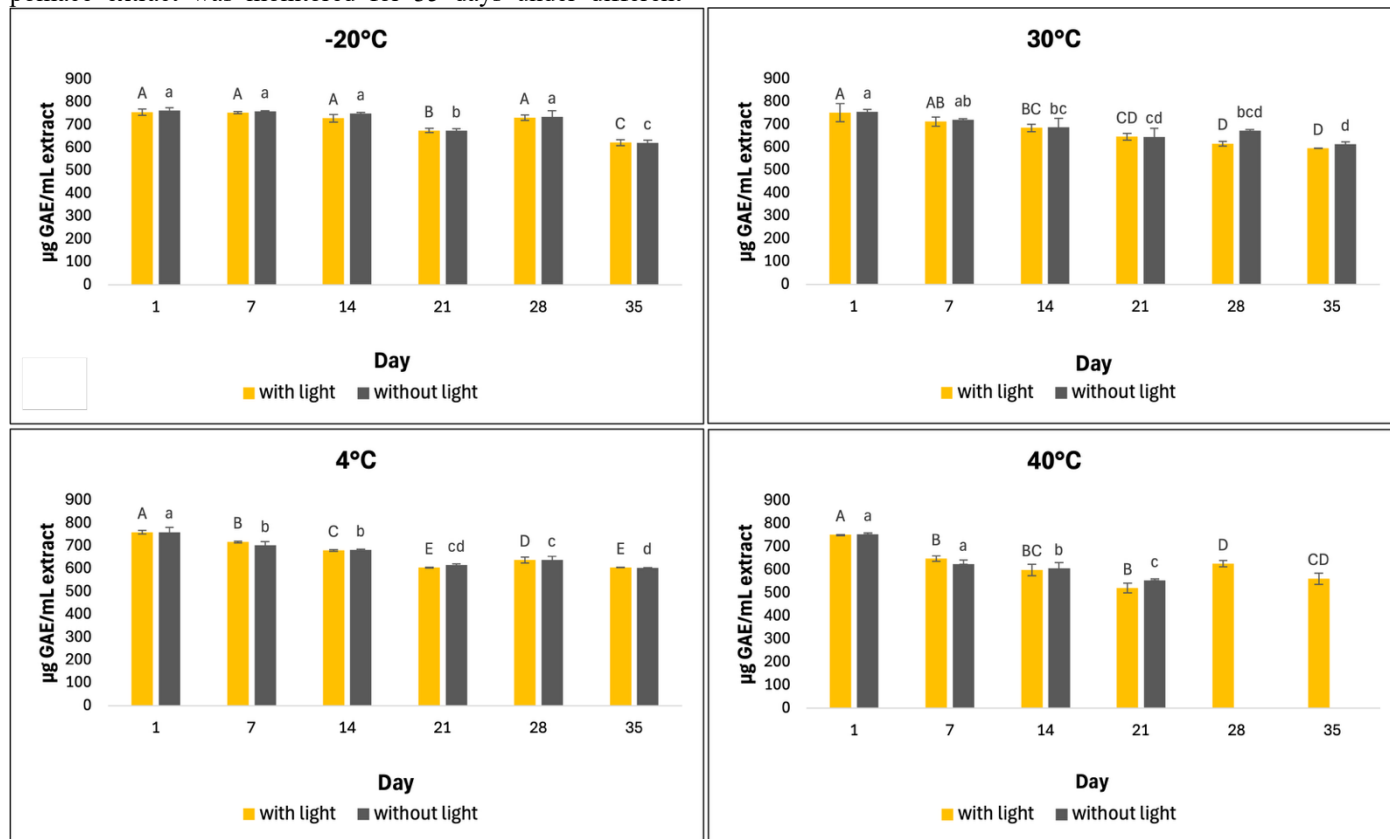


Fig.1. The total phenolic content of freeze-dried bignay pomace extract at different temperatures for 35 days. Error bars represent the standard deviation of means, n = 3. Different uppercase letters indicate significant differences ($p < 0.05$) among samples with light. Different lowercase letters indicate significant differences ($p < 0.05$) among samples without light. GAE refers to gallic acid equivalent. At 40°C (without light), analysis was discontinued on Day 28 due to mold growth.

The results revealed that TPC was significantly influenced by storage time. In general, TPC exhibited notable fluctuations throughout the 35-day period across all storage conditions, with an initial decline until Day 21, followed by an increase on Day 28 (except for extracts at 30°C with light exposure), and a final decline by the end of the period (Day 35).

At lower storage temperatures (-20°C and 4°C), phenolic degradation occurred at a slower rate. Extracts stored at -20°C showed no significant changes until Day 14, regardless of lighting condition, and consistently maintained the highest TPC throughout the study. In contrast, extracts stored at 40°C recorded the most significant reductions in TPC, with significantly lower concentrations at each time point. On Day 21, TPC levels in extracts stored at 40°C were as low as 509.49 µg GAE/mL (with light) and 540.76 µg GAE/mL (without light), whereas extracts stored at lower temperatures still maintained TPC levels of approximately 600 µg GAE/mL.

storage temperatures, with and without light exposure (Figure 1).

By Day 28, TPC further decreased in extracts stored at 30°C with light, while all other samples showed a temporary increase. However, for extracts stored without light, the experiment was discontinued after Day 28 due to mold growth. By Day 35, all samples exhibited a further decline in phenolic content.

Phenolic compounds are prone to degradation, as they are susceptible to hydrolysis and oxidation [21], often resulting in TPC reduction over time. However, some studies have reported unexpected increases in TPC at specific time points. Castro-López et al. [22] observed an increase in TPC in fruit beverages after 12 days of storage. Likewise, Puttongsiri and Haruenkit [23] found that the phenolic concentration in extracts of coated tangerine juice increased during the early stages of storage, before decreasing towards the end of a five-week storage period at various temperatures.

The increments in the concentration could be due to the formation of phenolic compounds from the breakdown of complex phenolic compounds. Kapcum and Uriyapongson [24] reported significant increases in the concentrations of protocatechuic acid and hydroxybenzoic acid in purple corn during storage. This increase was attributed to the degradation of anthocyanins and the subsequent formation of new phenolic compounds. Similarly, Gerardi et al. [25] found that phenolic compounds such as quercetin, quercetin 3-glycoside, and stilbenes increased over time in grape pomace flour, likely due to anthocyanin co-pigmentation interactions with flavonols.

In the case of *bignay*, which contains anthocyanins in the forms of malvidin, pelargonidin, and delphinidin [7-9], these compounds may have contributed to the observed increases in

total phenolic concentration during storage. A previous study on freeze-dried *bignay* pomace extracts found a considerable concentration of total monomeric anthocyanins at 474.89 mg cyanidin-3-glucoside equivalent (C3GE)/100 g DW [12]. Thus, the fluctuations in TPC could be attributed to the dynamic interactions of these anthocyanins and other phenolic compounds during the storage process.

Effect of temperature and lighting conditions on total phenolic content

The data presented in **Table 1** highlight the significant impact of temperature on TPC degradation in freeze-dried *bignay* pomace extract after 35 days of storage.

TABLE 1. COMPARISON OF THE TOTAL PHENOLIC CONTENTS OF FREEZE-DRIED *BIGNAY* POMACE EXTRACT AT DIFFERENT TEMPERATURES AND LIGHTING CONDITIONS

Sample Code	Temp. (°C)	Lighting condition	Total Phenolic Content (µg GAE/mL extract)		% reduction
			Day 1	Day 35	
-20L	-20	With Light	739.17 ± 13.22 ^a	608.40 ± 11.88 ^a	18
-20D		Without Light	744.79 ± 12.89 ^a	607.87 ± 10.91 ^a	18
4L	4	With Light	742.54 ± 8.27 ^a	591.36 ± 1.13 ^a	20
4D		Without Light	743.38 ± 19.39 ^a	590.62 ± 0.86 ^a	21
30L	30	With Light	734.96 ± 37.96 ^a	582.22 ± 1.28 ^a	21
30D		Without Light	738.05 ± 10.60 ^a	599.51 ± 10.54 ^a	19
40L	40	With Light	733.84 ± 2.71 ^a	548.89 ± 24.29 ^b	25
40D		Without Light	736.93 ± 5.52 ^a	-	-

Values are expressed as mean ± standard deviation, n = 3. Means with different lowercase superscripts indicate significant differences within columns ($p < 0.05$). At 40°C (without light), analysis was discontinued on Day 28 due to mold growth.

Lighting conditions had no significant effect on TPC ($p < 0.05$) at any temperature. At -20°C and 4°C, the TPC values of light-exposed and dark-stored samples were nearly identical. However, at 30°C, light-exposed samples showed a slight but statistically insignificant decrease, suggesting that light played only a minimal role in phenolic degradation compared with temperature-driven degradation.

These findings align with previous research, suggesting that temperature plays a more significant role in phenolic stability than light exposure. Deng et al. [26] found that higher temperatures accelerate oxidation and biochemical degradation of phenolics, leading to more rapid TPC losses over time. The rapid degradation observed at 40°C highlights the need for controlled storage conditions to preserve the functional properties of polyphenols. Selecting appropriate storage parameters, particularly time and temperature, is crucial for maintaining the integrity and potential applications of *bignay* pomace extract in various industries, including food, pharmaceuticals, and nutraceuticals.

Previous studies support these observations. Research by Del-Toro-Sánchez et al. [27] found that polyphenols from *Anemopsis californica* were more stable at lower temperatures, with up to 97% retention at 4°C and 20°C after 60 days. In contrast, temperatures as high as 50°C led to significant

degradation. Similarly, Esparza et al. [28] reported that Mazuelo grape stem extracts showed greater stability at 25°C compared to 40°C, and that light exposure worsened anthocyanin degradation at higher temperatures. These findings are consistent with the current study, where temperature played a more prominent role in phenolic stability than light.

In the present study, only temperature and lighting conditions were considered. However, the stability of phenolic compounds can be influenced by other factors, including their chemical structure, which may be prone to oxidation, hydrolysis, and polymerization when exposed to temperature fluctuations, humidity, oxygen, or metal ions [16,29]. As Ferreyra et al. [30] have noted, the presence of unsaturated bonds and hydroxyl groups in phenolic compounds increases their reactivity under certain environmental conditions. This leads to degradation pathways that compromise their antioxidant and bioactive properties. During storage, phenolic oxidation may occur enzymatically through the action of polyphenol oxidase or non-enzymatically via auto-oxidation in the presence of oxygen [31]. These processes convert phenolic molecules into highly reactive quinones, which subsequently undergo polymerization [32].

Degradation kinetics of total phenolics

The degradation of phenolics in *bignay* pomace extract across different temperatures and lighting conditions was calculated by performing regression analysis. Subsequently, the data were fitted to various kinetic models. **Table 2** presents the coefficient

of determination (R^2) and the reaction rate constant (k) obtained from fitting the data to the different reaction orders.

TABLE 2. KINETIC PARAMETERS OF PHENOLIC DEGRADATION OF *BIGNAY* POMACE EXTRACT AT DIFFERENT TEMPERATURES AND LIGHTING CONDITIONS

Lighting Condition	Temperature (°C)	Zero-order		First-order		Second-order	
		k	R2	k	R2	k	R2
With light	-20	3.2224	0.6524	0.0048	0.6453	0.00001	0.6379
	4	4.4025	0.8425	0.0067	0.8393	0.00001	0.8342
	30	4.5207	0.9887	0.0069	0.9921	0.00001	0.9934
	40	4.3737	0.5229	0.0070	0.5040	0.00001	0.4800
Without light	-20	3.4617	0.6447	0.0051	0.6376	0.00001	0.6303
	4	4.2055	0.8715	0.0064	0.8772	0.00001	0.8808
	30	3.6113	0.8732	0.0054	0.8718	0.00001	0.8684
	40*	ND	ND	ND	ND	ND	ND

*ND, not determined for extract at 40°C. Analysis was discontinued on Day 28.

R^2 – coefficient of determination

k – rate constant

From the results, the kinetic degradation of total phenolic content generally follows a zero-order reaction with a coefficient of determination ranging from 0.5229 to 0.9887. This observation suggests that, in most conditions, the rate of phenolic degradation was independent of the phenolic concentration. With zero-order reactions, the degradation occurs at a constant rate over time. The corresponding rate constants (k) for zero-order kinetics ranged from 3.2224 to 4.5207, depending on the storage condition. These values indicate the speed at which phenolic compounds degrade under each treatment, with higher k values representing faster degradation. Notably, the lowest rate constants were observed at -20°C, indicating that the degradation of phenolics occurred at the slowest rate under freezing conditions. Low temperatures were able to minimize oxidative and degradative reactions, likely due to reduced molecular kinetic energy and limited enzymatic or chemical activity.

These findings align with the principles of chemical kinetics, where lower temperatures reduce molecular energy, decreasing the likelihood of degradation reactions [29].

Previous studies have reported similar degradation behaviors in plant extracts. Eadmusik et al. [33] found that *Tiliacora triandra* leaf extract followed zero-order kinetics, while Zapata et al. [34] reported that the phenolic compounds in *Bixa orellana* L. leaves were degraded according to first-order kinetics. The investigators attributed the degradation mainly to oxidation and structural breakdown caused by heat. Likewise, Patras et al. [35] demonstrated that microencapsulated anthocyanins from freeze-dried lowbush berries degraded following first-order kinetics, with the rate of degradation being strongly dependent on temperature. For the antioxidants in

dried piper betel, a zero-order kinetic model best described its degradation kinetics [16]. Silva et al. [36] also found that the degradation in peanut flours followed both zero- and first-order kinetics due to oxidation, hydrolysis, and polymerization processes.

The observed temperature-dependent degradation of phenolic compounds in *bignay* pomace extract aligns with findings from other fruit pomace studies. Katsinas et al. [37] reported that phenolic compounds in olive pomace exhibited increased phenolic instability at higher temperatures, thereby reducing antioxidant potential. Similarly, Sharma et al. [38] demonstrated that phenolic extracts from berry pomace exhibited notable reductions in antioxidant capacity when exposed to elevated temperatures.

B. Antioxidant activity

DPPH radical scavenging activity

Figure 2 shows the changes in the DPPH radical scavenging activity of *bignay* pomace extract under varying temperatures and lighting conditions over a 35-day period. Initially, the scavenging activity remained stable from Day 1 to Day 7 across all conditions, suggesting that the *bignay* pomace extract retains its antioxidant properties early on regardless of environmental factors. This consistency suggests that the extract may be relatively stable during the first week of storage, regardless of whether it is subjected to low temperatures (4°C, -20°C) or higher temperatures (30°C, 40°C).

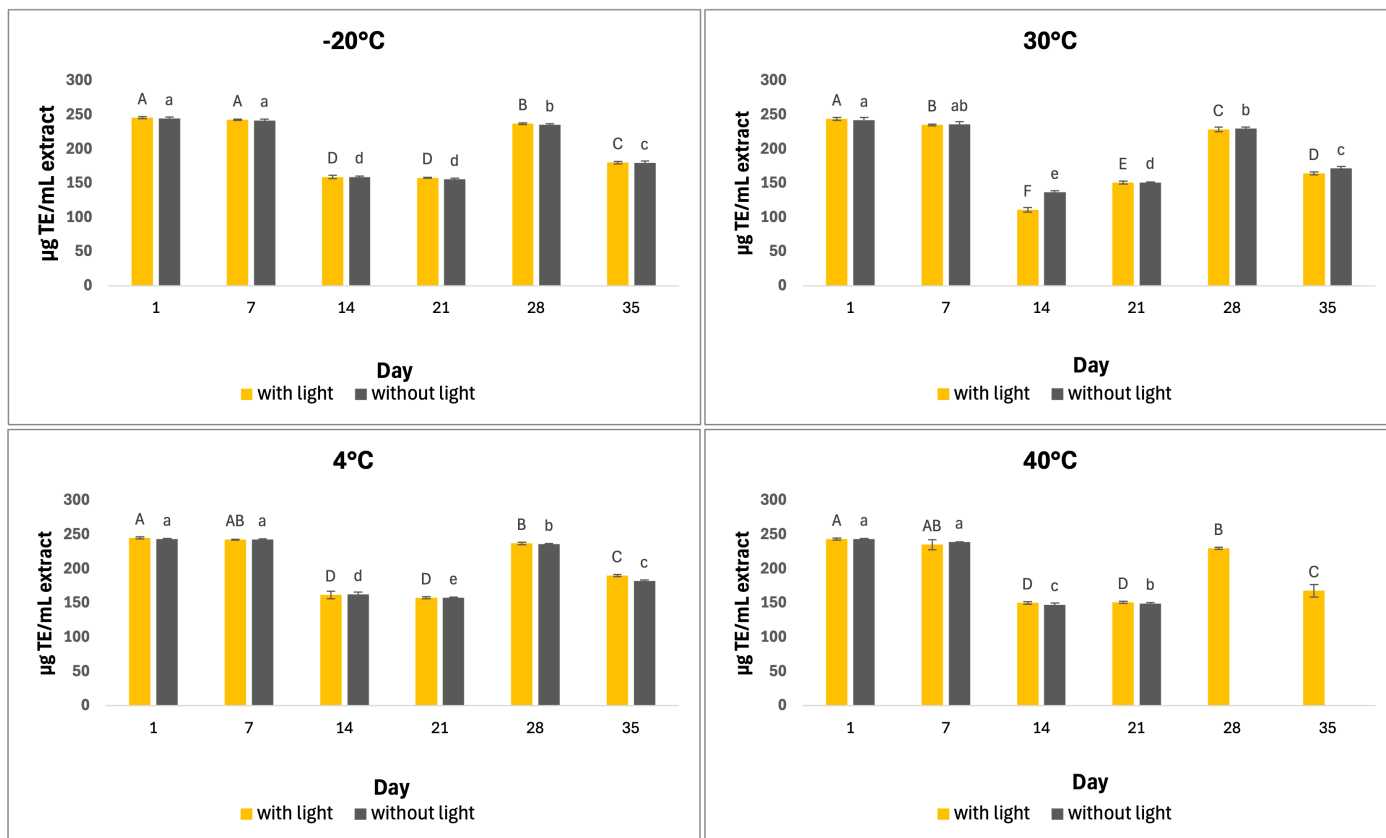


Fig. 2. DPPH radical scavenging activity of freeze-dried *bignay* pomace extract with different lighting conditions as affected by time. Error bars represent the standard deviation of means, n = 4. Uppercase letters indicate significant differences ($p < 0.05$) among samples with light. Lowercase letters indicate significant differences ($p < 0.05$) among samples without light. TE refers to Trolox equivalent. At 40°C (without light), analysis was discontinued on Day 28 due to mold growth.

By Day 14, a sharp decrease in antioxidant activity was observed. Reductions ranged from 33% to 53%, with extracts stored at lower temperatures (-20°C and 4°C) showing a progressive decrease up to Day 21, while those at 30°C and 40°C exhibited slight increases during the same period. As the storage period progressed, a recovery of 50–52% in scavenging activity occurred across all samples on Day 28. However, by Day 35, a notable decline in activity ($p < 0.05$) was observed. This late-stage reduction may reflect the eventual breakdown of antioxidant molecules due to prolonged exposure to varying storage conditions. It suggests that while there might be short-term stabilization or even enhancement in antioxidant activity, prolonged storage, especially under extreme conditions, leads to significant loss of activity.

Phenolic compounds are the primary contributors to the antioxidant activities of *bignay* pomace extracts. These compounds can scavenge free radicals, such as reactive oxygen species, due to their hydroxyl groups [39]. Based on the results,

the DPPH scavenging activity of *bignay* pomace extract decreased or increased in response to changes in total phenolic content. The significant increase in the DPPH scavenging activity after 28 days can be attributed to the increase in the phenolic concentration of the extracts, which also occurred on the 28th day of storage. However, the antioxidant power decreased thereafter, and this trend was similarly observed in the TPC of *bignay* pomace extract. This trend aligns with the claim of Yu et al. [40] that the antioxidant activity of phenolic compounds exhibits a concentration-dependent increase in scavenging rate. Observing the prominent effect of storage time at higher storage temperatures signifies that storing the extract at a lower temperature could help mitigate the reduction in its activity.

The DPPH radical scavenging activity of *bignay* pomace extract was compared after 35 days to analyze the effect of lighting conditions and temperature (Table 3).

TABLE 3 . COMPARISON OF THE DPPH RADICAL SCAVENGING ACTIVITY OF FREEZE-DRIED *BIGNAY* POMACE EXTRACT AT DIFFERENT TEMPERATURES AND LIGHTING CONDITIONS

Sample Code	Temp. (°C)	Lighting condition	DPPH Radical Scavenging Activity ($\mu\text{g TE/mL extract}$)		% reduction
			Day 1	Day 35	
-20L	-20	With Light	240.51 \pm 1.69 ^a	176.18 \pm 1.82 ^{bc}	27
-20D		Without Light	239.27 \pm 2.10 ^a	175.85 \pm 2.50 ^{bc}	28
4L	4	With Light	240.02 \pm 1.43 ^a	185.98 \pm 1.41 ^a	23
4D		Without Light	238.04 \pm 1.31 ^a	178.49 \pm 1.36 ^b	25
30L	30	With Light	238.53 \pm 2.04 ^a	160.98 \pm 2.07 ^d	33
30D		Without Light	236.80 \pm 4.26 ^a	168.14 \pm 2.45 ^{cd}	29
40L	40	With Light	238.16 \pm 1.63 ^a	164.39 \pm 9.10 ^d	31
40D		Without Light	236.68 \pm 0.94 ^a	-	-

Values are expressed as mean \pm standard deviation, n = 4 . Means with different lowercase superscripts indicate significant differences within columns ($p < 0.05$). At 40°C (without light), analysis was discontinued on Day 28 due to mold growth.

The DPPH radical scavenging activity differed significantly among treatments ($p < 0.05$) on Day 35, ranging from 160.98 to 185.98 $\mu\text{g TE/mL}$ of extract. Samples stored at -20°C and 4°C showed significantly higher activities than those at 30°C and 40°C. The highest activity was observed in the extract stored at 4°C with light (4L) at 185.98 $\mu\text{g TE/mL}$. This was closely followed by 4D, -20L, and -20D, whose values were not significantly different, indicating good retention of antioxidant activity at these low temperatures. In terms of lighting conditions, a significant difference between the extract with light and without light was observed only at 4°C. At -20°C and 30°C, samples at different lighting conditions shared common superscripts, demonstrating no significant influence of light under these temperatures. In contrast, samples stored at 30°C and 40°C declined to $< 170 \mu\text{g TE/mL}$ extract with reductions of 29 to 33% from Day 1.

This pattern suggests that temperature had a more pronounced impact than light exposure on the stability of antioxidant properties. Notably, extracts stored at lower temperatures showed better retention of antioxidant activity. For instance, more than 70% stability in DPPH radical scavenging activity was retained by extracts kept under cold storage conditions (-20°C and 4°C) over the 35-day period.

Overall, the trends observed in this study underscore the complex nature of antioxidant stability in *bignay* pomace extract, with temperature and time playing critical roles in determining the extract's effectiveness as a radical scavenger. The findings suggest that while lower temperatures may reduce the rate of degradation, longer storage times ultimately result in decreased efficacy, highlighting the need for careful consideration of both storage conditions and duration to preserve the antioxidant potential of such extracts.

Ferric reducing antioxidant power

Figure 3 illustrates the significant effect of storage time on the ferric reducing antioxidant power (FRAP) of freeze-dried *bignay* pomace extracts, showing a significant decline ($p < 0.05$) in antioxidant capacity over the 35-day monitoring period. Initially, the FRAP ranged from 2146.84 to 2636.64 $\mu\text{g TE/mL}$ of extract. However, FRAP showed a consistent downward trend up to Day 21, suggesting ongoing oxidative degradation or structural changes in antioxidant compounds. The most substantial loss was observed between Days 7 and 14, with reductions of up to 45%, depending on the storage condition. Notably, the sample stored at 40°C (with light) exhibited the steepest drop. By Day 21, FRAP values in almost all samples had decreased substantially.

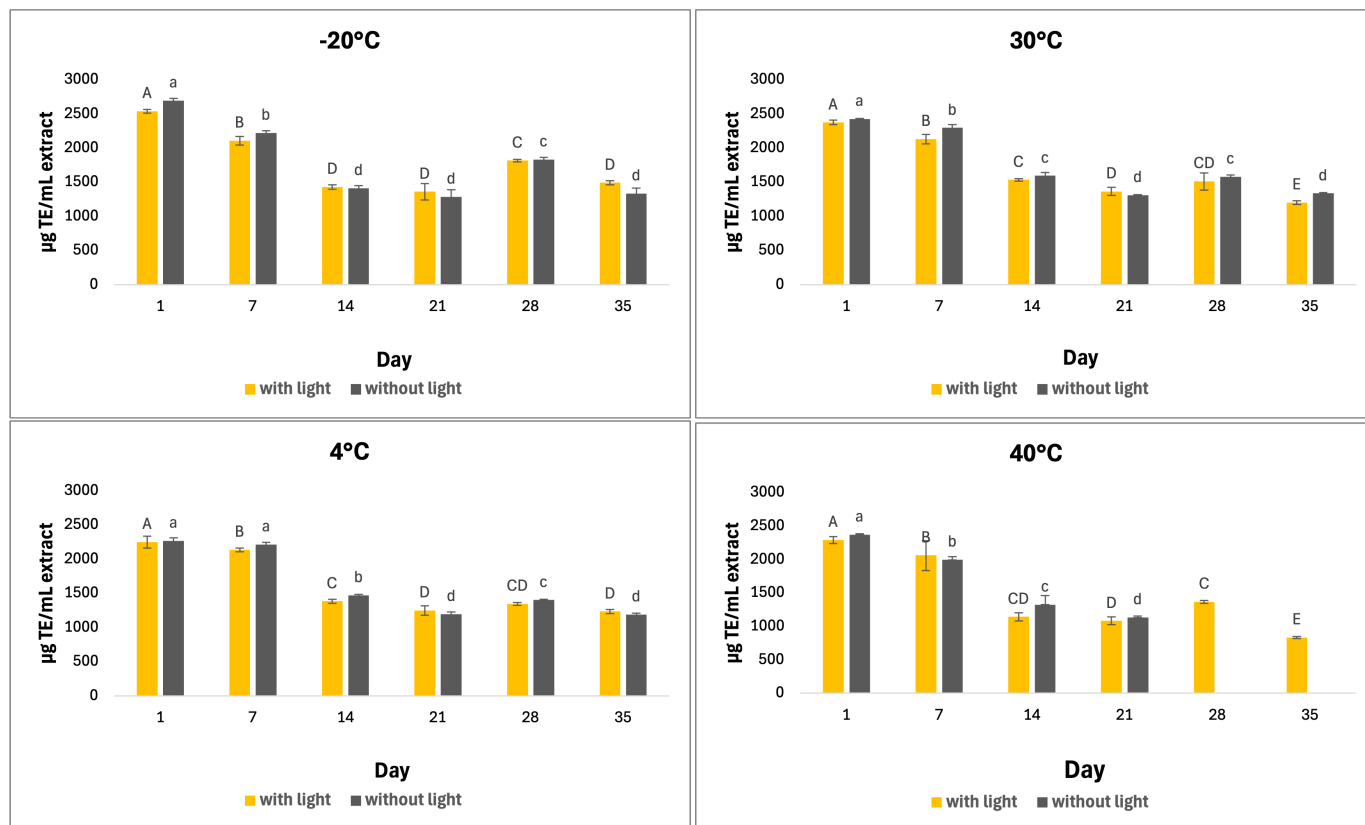


Fig. 3 . Ferric reducing antioxidant power of freeze-dried *bignay* pomace extract with different lighting conditions as affected by time. Error bars represent the standard deviation of means, n = 4. Uppercase letters indicate significant differences ($p < 0.05$) among samples exposed to light. Lowercase letters indicate significant differences ($p < 0.05$) among samples not exposed to light. TE refers to Trolox equivalent. At 40°C (without light), analysis was discontinued on Day 28 due to mold growth

Interestingly, the samples began to recover some of their antioxidant power on Day 28. The increase may be attributed to the release or transformation of bound phenolic compounds.

Notably, the extract stored at -20°C showed the highest FRAP level, exceeding 1700 µg TE/mL extract. This trend in the antioxidant activity highlights the effectiveness of low-temperature storage in preserving the bioactivity of phenolics present in freeze-dried *bignay* extract. However, by the final measurement on Day 35, the FRAP had significantly decreased

for all storage conditions, indicating the cumulative impact of the degradation process over time, regardless of the storage condition.

The impact of temperature and lighting conditions on the stability of *bignay* pomace extract's ferric reducing antioxidant power was further analyzed in Table 4, which showed that these factors significantly influenced the extract's stability.

TABLE 4 . COMPARISON OF THE FERRIC REDUCING ANTIOXIDANT POWER OF FREEZE-DRIED *BIGNAY* POMACE EXTRACT AT DIFFERENT TEMPERATURES AND LIGHTING CONDITIONS

Sample Code	Temp. (°C)	Lighting condition	Ferric Reducing Antioxidant Power (µg TE/mL extract)		% reduction
			Day 1	Day 35	
-20L	-20	With Light	2476.96 ± 24.42 ^a	1455.99 ± 31.693 ^a	41
-20D		Without Light	2636.64 ± 42.86 ^a	1299.03 ± 80.01 ^b	51
4L	4	With Light	2146.84 ± 70.80 ^a	1204.42 ± 29.20 ^c	44
4D		Without Light	2166.58 ± 70.80 ^a	1159.89 ± 17.99 ^c	46
30L	30	With Light	2267.05 ± 73.42 ^a	1173.25 ± 25.84 ^c	48
30D		Without Light	2363.93 ± 4.14 ^a	1304.60 ± 10.91 ^b	45
40L	40	With Light	2204.25 ± 40.33 ^a	812.59 ± 16.46 ^d	63
40D		Without Light	2324.46 ± 24.16 ^a	-	-

Values are expressed as mean ± standard deviation, n = 4. Means with different lowercase superscripts within a column indicate significant differences within columns ($p < 0.05$). At 40°C (without light), analysis was discontinued on Day 28 due to mold growth.

Throughout the 35-day storage period, significant differences were observed among treatments ($p < 0.05$). FRAP values ranged from 812.59 to 1455.00 $\mu\text{g TE/mL}$ extract, corresponding to losses of 41%–64%. The extract at -20°C (with light) had the highest reducing power, followed by the extracts at -20°C (without light) and 30°C (without light), whose values were not significantly different from each other. Samples 4L, 4D, and 30L had intermediate FRAP values ranging from 1173.25 to 1204.42 $\mu\text{g TE/mL}$ extract and did not differ significantly from one another. The extract stored at 40°C (with light) showed the most severe degradation in antioxidant power. Starting from a relatively high FRAP value on Day 1, the antioxidant power of this sample dropped to 812.59 $\mu\text{g TE/mL}$ extract by Day 35. This corresponds to a 63% reduction, further confirming that prolonged exposure to elevated temperature primarily leads to oxidative degradation or molecular instability of the bioactive phenolics present. In contrast, the extract stored at -20°C (with light) exhibited the least degradation. This further supports the observations in TPC and DPPH radical scavenging activity that lower temperatures are more conducive to preserving antioxidant activity in *bignay* pomace extract.

These findings are consistent with previous studies, such as the work by Serea et al. [41], which observed a similar pattern of antioxidant degradation in lettuce stored over time. In that study, the antioxidant activity dropped by as much as 63.15% between Day 6 and Day 15, highlighting the susceptibility of antioxidants to temperature and storage conditions.

The observed decrease in FRAP can be attributed to the breakdown of polyphenolic compounds, which are responsible for the antioxidant activity in *bignay* pomace. As previously noted, *bignay* pomace is rich in polyphenols, which act as antioxidants by donating electrons, hydrogen atoms, or by chelating metal ions [42]. The FRAP assay, which measures the reduction of Fe(III)-TPTZ to Fe(II)-TPTZ, directly correlates with the availability of these bioactive compounds. As storage time progressed and total phenolic content (TPC) declined, a corresponding decrease in FRAP was observed. However, the increase in TPC on certain days (such as Day 28) suggests that biochemical processes during storage might have led to the formation or release of additional phenolic compounds, which in turn contributed to the observed recovery in FRAP during that period.

In summary, storage time, temperature, and light conditions significantly impacted the antioxidant stability of freeze-dried *bignay* pomace extracts. While high temperatures, especially at 40°C , caused the most significant degradation, low temperatures helped preserve antioxidant activity. At higher temperatures, oxidative and hydrolytic reactions are promoted, leading to the breakdown of phenolic structures. Light exposure can further intensify these losses, as photo-oxidation induces the excitation of polyphenol molecules, leading to the formation of highly reactive radicals, causing structural degradation [43].

The Pearson correlation analysis revealed a statistically significant positive relationship between total phenolic content (TPC) and antioxidant activities in freeze-dried *bignay* pomace extract. Specifically, a moderate positive correlation was observed between TPC and DPPH radical scavenging activity ($r = 0.557$, $p < 0.001$) while a significantly large positive relationship was found between TPC and FRAP ($r = 0.819$, $p < 0.001$). These findings suggest that phenolic compounds are major contributors to the extract's antioxidant potential. The stronger correlation between TPC and FRAP indicates a more direct link between phenolic concentration and the extract's electron-donating capacity, which is the basis for the FRAP assay. In this context, the high correlation underscores the role of phenolic compounds as potent reducing agents capable of stabilizing free radicals by donating electron, rather than merely hydrogen atoms. The stronger correlation between TPC and FRAP, as compared to DPPH, suggests that the antioxidant mechanism in *bignay* pomace extract is predominantly reductive in nature rather than radical scavenging.

Similar results were reported by Liu et al. [44] in fruit vinegars and Muflihah et al. [45] in *Zingiberaceae* herbs, where a higher correlation between TPC and FRAP indicated that phenolic compounds predominantly act as electron donors.

The moderate correlation between TPC and DPPH scavenging activity suggests that while phenolic compounds contribute significantly to antioxidant activity, they are not the sole contributors to the extract's free radical scavenging activity. This implies the involvement of other bioactive constituents in the scavenging mechanism of freeze-dried *bignay* pomace extract.

This is supported by the observations of Esparza et al. [28], who reported that non-phenolic compounds such as ascorbic acid and carotenoids influenced the DPPH scavenging in plant extracts, which led to the weaker relationship between TPC and DPPH scavenging activity.

Further supporting this explanation, Yu et al. [40] demonstrated that the radical scavenging capacity of phenolics can vary based on concentration and their molecular structure. Structural interactions, such as conjugation and polymerization, can also influence the antioxidant capacity of individual phenolics. As a result, even extracts with similar TPC may differ in their actual radical scavenging activity depending on the specific composition and interaction of the phenolic compounds present. Overall, these findings confirm the effectiveness of phenolic compounds present in *bignay* pomace as reducing agents, making them valuable in applications requiring strong metal-reducing activity, such as food preservation, stabilization of lipid-rich products, and nutraceuticals aimed at combating oxidative stress.

The reductions in DPPH radical scavenging activity (Table 3) and FRAP values (Table 4) are consistent with the degradation kinetics described in Table 2. The zero-order rate constants (K) observed in the degradation of phenolics align with the decline

in antioxidant activity observed across all storage conditions. The extracts stored at higher temperatures, which exhibited greater rate constants for phenolic loss, also showed the most significant reductions in DPPH radical scavenging activity and FRAP values.

While these results highlight the promising potential of *bignay* pomace extract, the present study was limited to a 35-day storage period. Likewise, it used a relatively small sample size, which may affect the generalizability of the findings. The 35-day duration was selected to observe short-term degradation patterns commonly assessed in initial stability studies of phenolic extracts. This duration allowed the identification of temperature- and light-dependent degradation trends. This work thus provides baseline information on the stability of *bignay* pomace extract, which can guide future upscaling and long-term storage investigations. However, further studies with larger sample sets and longer storage periods are needed to validate these findings. Microbiological assessments are also recommended to evaluate potential spoilage risks during storage.

IV. CONCLUSION

This study investigated the impact of storage conditions on the TPC and antioxidant activity of freeze-dried *bignay* pomace extract. The results indicated that temperature and storage duration significantly influenced both parameters, whereas light had a comparatively minor effect. Over the 35-day storage period, TPC and antioxidant activity fluctuated but showed an overall decline across all storage conditions, and the greatest losses occurred at 40°C. Phenolic degradation generally followed zero-order kinetics, with the lowest rate constants at -20°C. The strong positive correlations between TPC and both DPPH radical scavenging activity and FRAP confirm that phenolics are the main contributors to antioxidant activity.

Based on these findings, low-temperature storage (freezing or refrigeration) provided the best preservation of phenolic compounds, while higher temperatures (40°C) led to accelerated degradation, confirming that temperature played the most significant role in maintaining bioactive stability.

The 35-day storage period provided valuable baseline information on the short-term degradation of the extract, serving as a foundation for future upscaling and long-term stability assessments. Longer-term studies are recommended to confirm these trends and evaluate real-world storage performance.

These findings suggest that maintaining low-temperature and light-protected storage can help preserve the bioactive properties of *bignay* pomace extract. With further validation, such stability may support its application as a natural preservative and functional ingredient in food, nutraceutical, and active packaging applications.

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CONFLICT OF INTEREST

Authors declare no conflict of interest to disclose.

Declaration of generative AI in the scientific writing process. During the preparation of this work, the authors used ChatGPT in order to check for spelling and grammar and to enhance readability. The authors have reviewed and edited the content as needed. The authors will take full responsibility for the content of the published article.

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