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Healthy Drink Mixtures With Tropical Fruits, Cashew (*Anacardium occidentale*), Acerola (*Malpighia emarginata*), and Aloe Vera (*Aloe barbadensis* Miller)

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ABSTRACT

Tropical fruits are sources of bioactive compounds with potential health benefits. The objective of this study was to optimize functional beverage formulations using *Aloe vera* gel (*Aloe barbadensis* Miller), clarified cashew juice (*Anacardium occidentale*), and acerola pulp (*Malpighia emarginata*), integrating mixture design optimization with kinetic shelf-life modeling and explicit control of aloin content, an approach not previously reported for tropical fruit–aloe beverages. A D-optimal mixture design was applied to determine the proportions of fruit juice, water, and sweetener, keeping the content of aloe gel and stabilizing agent constant. Physicochemical analyses included pH, titratable acidity, total soluble solids, and ascorbic acid content. High-performance liquid chromatography (HPLC) was used to quantify ascorbic acid and aloin. The physicochemical characteristics presented values within previously published ranges. The optimized cashew and acerola beverages showed an ascorbic acid content of up to 158 mg/100 mL of beverage, aloin levels below 0.0012 μg/mL in the final beverage, and desirable physicochemical stability. The estimated shelf life of the beverages was 51 days, and their stability followed first-order kinetics, with greater degradation observed at 60°C. This study provides a novel formulation strategy by integrating *Aloe vera* gel with tropical fruit juices through a D-optimal mixture design, combined with analytical control of aloin and kinetic-based shelf-life prediction. The results contribute original data on the stability and safety of *Aloe vera*–based functional beverages, supporting their agroindustrial application using tropical fruits from the Colombian Caribbean, aligning with current consumer trends toward healthier food alternatives and promoting agroindustrial innovation.

1 | Introduction

Functional products represent one of the fastest-growing segments of the food industry, driven by consumer demand for products that provide health benefits beyond basic nutrition [1]. These beverages commonly incorporate plant-derived ingredients rich in bioactive compounds such as vitamin C, polyphenols, carotenoids, and polysaccharides, which are associated with antioxidant, anti-inflammatory, and immunomodulatory effects. However, the development of stable functional beverages based on natural matrices remains a significant technological challenge. Bioactive compounds are often physicochemically unstable in aqueous systems, highly susceptible to oxidation, and difficult to preserve during processing and storage while ensuring product safety and quality.

The northern region of the Colombian Caribbean, thanks to its diversity of microclimates, high relative humidity, and favorable

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temperatures, is an optimal environment for the cultivation of these species. This privileged agroecological condition not only favors the production of quality raw materials but also provides opportunities for their agroindustrial use, especially in the production of juices, nectars, and fruit and vegetable blends.

Tropical fruits constitute an important source of bioactive compounds and have gained increasing attention for functional beverage applications. Acerola (*Malpighia emarginata*) is recognized as one of the richest natural sources of ascorbic acid, with concentrations substantially higher than those found in conventional citrus fruits [2]. Despite its nutritional value, the high vitamin C content of acerola also poses a challenge due to its pronounced instability under typical processing and storage conditions [3]. Similarly, cashew apple (*Anacardium occidentale*) provides vitamin C, flavonoids, carotenoids, and phenolic acids, along with a favorable sensory profile that supports its incorporation into juice blends [4–6]. Nevertheless, variations in composition and oxidative sensitivity can affect the stability and quality of cashew-based beverages.

In parallel, *Aloe vera* (*Aloe barbadensis* Miller) has attracted considerable interest in the formulation of functional foods due to its polysaccharides, polyphenols, and other phytochemicals associated with antioxidant, antimicrobial, and digestive health benefits [7, 8]. Despite its functional potential, the incorporation of *Aloe vera* into beverage systems presents a critical safety and technological limitation: the presence of anthraquinones such as aloin [9]. These compounds are associated with undesirable sensory attributes and are subject to strict toxicological regulations. Consequently, the development of *Aloe vera*-based beverages requires not only appropriate formulation strategies to preserve beneficial compounds but also rigorous analytical control to ensure the reduction of aloin to safe trace levels.

In fruit-based beverages, the stability of vitamin C is strongly influenced by formulation, processing, and storage conditions. Its degradation generally follows first-order kinetics and is highly dependent on temperature [10, 11], making kinetic modeling an essential tool for predicting quality loss and shelf life. However, many studies on tropical functional beverages focus separately on formulation optimization, analytical characterization, or shelf-life evaluation, without integrating these aspects into a unified methodological framework.

Furthermore, limited information is available on the combined use of tropical fruit juices and clarified *Aloe vera* gel in beverage formulations optimized using mixture design methodologies, together with chromatographic validation of bioactive compounds and kinetic modeling of ascorbic acid degradation under realistic storage conditions. The lack of integrated approaches restricts the ability to simultaneously address formulation efficiency, safety compliance, and shelf-life prediction [12].

Therefore, an integrated strategy is required that concurrently considers formulation optimization, analytical control of aloin and ascorbic acid, and kinetic modeling of vitamin C degradation. Such an approach is essential for the development of stable, safe, and functional aloe-based tropical beverages.

The objective of this study was to optimize functional beverage formulations based on clarified *Aloe vera* gel combined with cashew and acerola juices using a D-optimal mixture design, incorporating validated high-performance liquid chromatography (HPLC) analysis for aloin and ascorbic acid, and applying kinetic models to predict shelf life under storage conditions.

2 | Materials and Methods

2.1 | Plant Material

Acerola fruits (red), cashew pseudofruits (red and yellow), and *Aloe vera* leaves for obtaining *Aloe vera* gel were harvested in the cultivation area of the Department of Córdoba at optimal maturity based on visual appearance and total soluble solids (°Brix). Cashew pseudofruits were harvested in the village of Pisa Bonito, municipality of Chinú (Córdoba), while *Aloe vera* leaves, approximately 2 years old, were collected in the same region. After harvest, all raw materials were transported to the vegetable pilot plant at the University of Córdoba, Berástegui campus (Ciénaga de Oro, Córdoba; 8°40′26″ N, 75°46′44″ W) and processed within 24 h under refrigerated conditions (4°C) to minimize postharvest deterioration.

2.2 | Reagents

Sodium hydroxide (Merck), phenolphthalein (Merck), oxalic acid (Sigma-Aldrich), 2,6-dichlorophenolindophenol (Sigma-Aldrich), pectinase enzyme (Sigma-Aldrich), activated carbon (Sigma-Aldrich), citric acid (Sigma-Aldrich), sodium citrate (Merck), calcium lactate (Sigma-Aldrich), gellan gum (Merck), ascorbic acid (Sigma-Aldrich), and aloin (Merck) were used in this study. Reagents used for physicochemical analyses were of analytical grade, while solvents and standards used for HPLC analyses were of appropriate chromatographic grade, according to the requirements of each method.

2.3 | Obtaining Raw Materials

2.3.1 | Acerola Juice

Ripe, healthy acerola fruits were blanched by immersion in water at 99°C–100°C for 30 s and then processed in a refiner. The pH of the pulp obtained was adjusted to 4.9, and pectinase enzyme was added at a ratio of 1 mL per 10 L of juice for 5 min. The enzyme was inactivated by heating to 69°C–70°C. The juice was then clarified by filtration using a Corel 234 Model 2009 filter press equipped with multifilament polypropylene mesh and 0.45-μm pore size filter paper. The clarified juice was hermetically sealed in 2-L containers and stored at 4°C for subsequent analysis and beverage production following pectinase-assisted juice clarification approaches previously reported for fruit juices [13].

2.3.2 | Cashew Juice

The cashew apple kernels were separated beforehand, and the unpeeled pseudofruits were blanched in water at 100 C for 3 min. They were then left to rest at room temperature (29°C–30°C) before proceeding with the extraction of the whole juice using manual juicers. The extracted juice was collected in previously cleaned and disinfected plastic containers. Subsequently, clarification was performed by filtration in a Corel 234 Model 2009 filter press, equipped with multifilament polypropylene mesh and cellulose paper with a pore size of 0.45 μm. The clarified cashew juice was packaged in 5-L containers and stored under refrigeration (4 C) until further physicochemical analyses and

beverage production, following standard laboratory-scale juice processing approaches reported for tropical fruits [13].

2.3.3 | *Aloe vera* Gel and Cubes

The *Aloe vera* leaves were stripped, the roots were removed, and they were washed with drinking water to eliminate organic residues. The outer rind was manually removed using knives to obtain *Aloe vera* fillets. The inner parenchymatous gel was then washed, peeled, and cut into approximately 1-cm-thick pieces using a mandolin. The cubes obtained were washed with drinking water, wrapped in mesh-type cloth, and sterilized in an autoclave at 121°C for 15 min. They were then refrigerated until used in the preparation of beverages. The yield of cubes from the leaf was 11%. The cubes were processed in an industrial blender until a viscous consistency was obtained, with small cubes suspended and a slightly yellowish-reddish color, attributable to the residual presence of anthraquinones. This mixture was heated in a pot at 37°C, pectinase was added at a ratio of 1 mL/10 L of gel, and it was kept for 5 min.

The mixture was then heated to 70°C for 30 min to inactivate the enzyme. At this stage, activated carbon (15 g/L) was added to remove aloin, an anthraquinone compound responsible for undesirable taste and odor and subject to strict regulatory control. According to current safety assessments, aloin and related hydroxyanthracene derivatives should be absent or present only at trace levels in food products due to their potential toxicological effects [14]. The processing of *Aloe vera* gel, including filleting, enzymatic treatment, clarification, and thermal stabilization, followed established quality and safety guidelines widely reported in the literature [15, 16]. The gel underwent double filtration using a filter press. In the first stage, silica was used as an adjuvant to retain the activated carbon. The pH was then adjusted to a range of 3.3–3.5, and a second filtration was performed using a cellulose membrane to remove any remaining residues. The clarified gel obtained was transparent, shiny, and had adequate viscosity. The pH of the clarified *Aloe vera* gel was adjusted using food-grade citric acid until the desired pH range was reached. pH measurements were performed using a calibrated pH meter. The gel was packaged in 2- to 5-L containers and stored under refrigeration for subsequent analysis and beverage production.

2.4 | Physicochemical Analysis of Raw Materials and Beverages

The pH and total acidity values of the raw materials and beverages were determined using methods 981.12 and 942.1, respectively [17]. A pH meter (Hanna Instruments, United States) was used for this purpose. The samples were conditioned in distilled water at a ratio of 1:10 (sample:water) and then titrated potentiometrically with 0.1 N NaOH. In addition, soluble solids were measured using digital refractometry (Atago, Japan), with the results expressed in degrees Brix [17]. The organic acid content was expressed in terms of the percentage of citric acid per 100 mL of the drink.

2.5 | Formulation and Preparation of Beverages

Two *Aloe vera* beverages were formulated by adding fruit juice (acerola or cashew) [18]. A D-optimal mixture design was used to determine the optimal ratio of the beverage components, which included fruit juice (A), water (B), and sweetener (C). The combined fraction of these ingredients represented 69.8% of the total formulation. The ratio between components A, B, and C varied according to the established experimental design, based on mass balances defined by (1). The remaining 30.2% corresponded to a constant component (R), composed of *Aloe vera* gel (gel and cubes), additives, and stabilizers, which remained unchanged in all formulations. Specifically, the aloe fraction consisted of 25% clarified *Aloe vera* gel and 5% *Aloe vera* gel cubes (5:1 w/w ratio), and this proportion was maintained constant throughout all formulations. The concentrations (% *w*/*w*) of the mixture components and the level limits are presented in Table 1. According to mixture design theory, the sum of the proportions of the mixture components must remain constant. In this study, a D-optimal mixture design was applied, in which fruit juice (A), water (B), and sweetener (C) constituted the variable components of the formulation. The total proportion of these components was fixed at 69.8% (*w*/*w*), as expressed in (1):

TABLE 1 | Factors and levels in the mixture design.

Factors (%(*w*/*w*))	Cashew		Acerola	
	Min.	Max.	Min.	Max.
Fruit juice	10.012	18.000	12.002	19.995
Water	45.220	52.528	42.615	50.239
Sweetener	6.600	7.280	7.203	7.579

$$\text{fruit juice}(A) + \text{water}(B) + \text{sweetener}(C) = 69.8\%, \quad (1)$$

where A, B, and C represent the mass fractions (% *w*/*w*) of fruit juice, water, and sweetener, respectively.

Restrictions were necessary to control the soluble solid content in the resulting beverages. To control the soluble solid content of the final beverages, a restriction was applied based on the contribution of soluble solids from the fruit juice and the sweetener. This restriction ensured that the total soluble solids remained within the desired sensory range, as described in (2):

$$8.1 < \text{SSjuice} + \text{SS} - \text{sweetener} < 8.2, \quad (2)$$

where SSjuice represents the soluble solid contribution from the fruit juice and SSsweetener represents the soluble solid contributed by the added sweetener.

The *Aloe vera* gel and fruit juice were mixed in a clean container. A combination of sucrose and fructose in a 70:30 ratio was used as a sweetener, which was dissolved in the juice mixture. At the same time, water was heated to 90°C and citric acid, sodium citrate, and calcium lactate were added to promote the suspension of the cubes. Once these compounds were dissolved, gellan gum was added as a stabilizer. The resulting solution was mixed with the juices and stirred carefully to homogenize it and prevent the formation of lumps. Finally, ascorbic acid and sterilized *Aloe vera* gel cubes (solid gel pieces, approximately 0.5 cm^3) were added.

The formulated beverages were subjected to a thermal treatment using a plate pasteurizer (INOXPA 2000). The temperature was automatically increased to 72°C and immediately reduced to 4°C, ensuring rapid heating and cooling to minimize thermal degradation of bioactive compounds.

2.6 | Evaluation of Bioactive Components and Beverage Stability

2.6.1 | Ascorbic Acid

The identification and quantification of ascorbic acid in the raw material and beverages were performed by HPLC, following the previously reported procedure [19], with modifications adapted to the characteristics of the matrix under study. For this purpose, an HP 1100 series liquid chromatography system (Agilent Technologies) was used, equipped with a G1322A degasser, a G1311A quaternary pump, and a G1314A UV-Vis detector. Chromatographic separation was performed using a Hypersil ODS C18 column (5 μm, 4.0 mm × 250 mm). Sample preparation included solid–liquid extraction. The procedure consisted of homogenizing the sample with 0.05 N sulfuric acid and 0.05 N phosphoric acid, followed by centrifugation at 6000 rpm for 20 min to facilitate separation of the aqueous phase. Subsequently, the aqueous extract was filtered, measured, and injected into the chromatograph in triplicate. The samples were analyzed at 25°C. Approximately 20 μL of the sample was injected.

Quantification was performed using an external standard solution of ascorbic acid (50 mg/L in 0.05 N phosphoric acid). Method validation was assessed based on calibration curve linearity ($R^2 > 0.997$), repeatability of triplicate injections, and consistency of retention times, ensuring reliable quantification of ascorbic acid in both raw materials and final beverages. Based on previous reports [20–22] and with the aim of optimizing the resolution and sensitivity of the chromatographic signal, different mobile phases were evaluated. The best separation was obtained using a mobile phase composed of water–methanol–acetonitrile (74.4:25.0:0.6) adjusted to pH 2.7 with 0.03 M KH_2PO_4 buffer solution. Detection was performed at 254-nm wavelength with the highest response for ascorbic acid. Representative chromatograms for ascorbic acid identification and quantification were obtained under the described HPLC conditions, confirming peak purity and consistent retention times across all analyzed samples.

2.6.2 | Aloin

The aloin content in bitter aloe juice (used as a processing intermediate) and in the final beverages formulated with *Aloe vera* combined with fruit juices (acerola and cashew) was determined by HPLC, following previously validated methodological principles for *Aloe vera* extracts [23, 24]. The analysis was performed on an Accela HPLC chromatograph (Thermo Fisher Scientific Inc., United States), equipped with an Accela 600 pump and a diode array detector (DAD, UV/Vis). A Hypersil Gold C18 column (50 × 2.1 mm, 1.9 μm) was used for separation, and data processing was performed using ChromQuest 5.0 software.

The samples were analyzed in triplicate at 25°C, with an injection volume of 15 μL. The mobile phases were as follows: mobile phase A, composed of water–methanol–acetonitrile (74.4:25.0:0.6, *v*/*v*/*v*); detection was performed at a wavelength (λ max) of 252 nm. The column temperature was maintained at 40°C, with a flow rate of 0.95 mL/min. The elution gradient was as follows: 0 min (92% A), 2.5 min (68% A), 3.0 min (92% A), and 4.0 min (92% A).

Aloin quantification was performed using the external standard method, employing a calibration curve constructed with standard solutions ranging from 5 to 100 mg/L. Data validation was carried out by evaluating calibration linearity ($R^2 = 0.997$), repeatability of triplicate analyses, and signal stability, ensuring accurate determination of aloin in intermediate extracts and final beverages. The concentration of the analyte in the samples was calculated using the ratio between the chromatographic area (mAU ∗ min) and the response factor. Finally, the aloin content in the beverages was expressed in mg/L. All measurements were performed in triplicate.

2.6.3 | Optimization and Stability

A numerical optimization was performed on the results of the variables of the developed beverages, considering the following criteria of influence of the response variables: pH (minimum), soluble solids (maximum), acidity (in the range), and ascorbic acid content (maximum). The two selected formulations were prepared. The stability of the ascorbic acid and the shelf life were evaluated. To evaluate the stability of ascorbic acid in beverages formulated with cashew and acerola, it was evaluated during a 60-day storage period under two controlled conditions: refrigeration (4°C) and elevated room temperature (32°C). The determinations were made on Days 1, 10, 20, 40, and 60 by HPLC, under the conditions described above [25].

The shelf life of the beverages was estimated based on the loss of ascorbic acid, a key quality parameter. The degradation kinetics were adjusted to a first-order model, widely accepted to describe degradation processes of bioactive compounds in beverages [26], according to the following expression:

$$\ln X = \ln X_o - \mathrm{kt}, \tag{3}$$

where X is the concentration of ascorbic acid (mg/100 mL of beverage), X_o is the initial concentration, k is the degradation rate constant (d^{-1}), and t is time (days).

The relationship between the rate constant and temperature was described by the Arrhenius equation (Equation (4)), in accordance with recent studies on the kinetics of vitamin C degradation in beverages during storage [26]:

$$k_{(T)} = Ae^{-((\mathrm{Ea})/(\mathrm{RT}))}, \tag{4}$$

where k is the rate constant (d^{-1}), T is temperature (K), Ea is the activation energy ($J \cdot mol^{-1}$), A is the frequency factor, and R is the universal gas constant (8.314 J/mol·K). These equations allowed us to estimate the half-life of beverages at different temperatures. All kinetic determinations were performed in triplicate.

Kinetic fitting of ascorbic acid degradation data was performed using Design-Expert software (Version 13, Stat-Ease Inc., Minneapolis, MN, USA), applying a first-order kinetic model and Arrhenius equation.

2.7 | Statistical Analysis

The data are presented as mean ± standard deviation (SD). The independent variables were the percentage of juice (A), water (B), and sweetener (C) in the ranges specified in Table 1. The response variables included pH, soluble solids (°Brix), titratable acidity, and ascorbic acid content (mg/100 mL of beverage) for both acerola and cashew. Normal distribution and homoscedasticity were verified with the Kolmogorov–Smirnov and Bartlett tests, respectively. Significant differences between means were determined using ANOVA. Statistical analyses were

TABLE 2 | Physicochemical characterization of raw material.

Parameters	*Aloe vera*	Cashew	Acerola
pH	5.86 ± 0.04	4.13 ± 0.03	3.60 ± 0.02
Acidity (g ascorbic acid/100 g pulp)	Not determined	0.03 ± 0.09	1.01 ± 0.06
Soluble solids (°Brix)	5.84 ± 0.03	8.89 ± 0.08	9.97 ± 0.03

performed using GraphPad Prism and Design-Expert, Version 8.0.6 (Stat-Ease, Inc., Minneapolis, MN, USA). Analyses were performed with a significance level of $p < 0.05$.

3 | Results and Discussion

3.1 | Physicochemical Characterization of the Raw Material

The physicochemical parameters determined in the pulp of *Aloe vera*, cashew, and acerola are presented in Table 2. The pH values obtained for *Aloe vera* (5.86 ± 0.04), cashew (4.13 ± 0.03), and acerola (3.60 ± 0.02) are within the acidic range characteristic of tropical fruits. Previous studies have reported similar values of 5.60 for *Aloe vera* [27], 4.8 for cashew [28], and 3.7 for acerola [29]. This characteristic variable contributes favorably to the microbiological stability of beverages made from these matrices, since acidic environments limit the growth of spoilage and pathogenic microorganisms [30].

Regarding total acidity, expressed as grams of ascorbic acid per 100 g of pulp, values of 0.03 ± 0.09 were obtained for cashews and 1.01 ± 0.06 for acerola. The high acidity of the latter is consistent with its high vitamin C content, which in fresh fruit can reach between 1500 and 4500 mg/100 g, conferring notable antioxidant and functional properties in processed matrices [31, 32]. In contrast, cashew presented considerably lower acidity, consistent with reports for the Colombian varieties Regional 8315 and Mapiria, which exhibit low levels of organic acids, with sugars and polyphenols predominating in their composition [33].

Soluble solids, expressed in degrees Brix (°Brix), presented values of 5.84 ± 0.03 for *Aloe vera*, 8.89 ± 0.08 for cashew, and 9.97 ± 0.03 for acerola. These compounds reflect the presence of sugars and other solutes that significantly influence sensory perception, particularly sweetness and beverage acceptability. In previous studies, values of 5.5–14.5°Brix were reported in cashew juice [28, 34, 35], which demonstrates the variability associated with factors such as origin and extraction method. For acerola, they reported a value of 7.8°Brix in the pulp [36], which is consistent with typical values for this species.

3.2 | Physicochemical Characteristics of the Beverages

Beverages enriched with cashew juice had pH values ranging from 3.56 to 3.77, with an average of 3.67 ± 0.0484. These data showed a significant fit to a quadratic model for the linear mixture and binary combinations of the components, with the R2 explaining 82% of the variation. The ascorbic acid content ranged between 17.0 and 36.0 mg/100 mL of beverage, with a mean value of 26.2 ± 5.0 mg/100 mL. A linear mixture model based on component proportions explained 69.6% of the variability ($R^2 = 0.696$). Meanwhile, soluble solids revealed results between 8.17 and 9.8, with an average of 9.17 ± 0.48; however, they did not present a model that explained the behavior of the variable despite being a variable that influences the acceptability and shelf life. Regarding acidity, the data obtained were in the range of 0.17–0.36 with a mean of 0.26 ± 0.05, with no effects of the components or the proportions in which they are mixed. The equations of the graphs for pH and ascorbic acid of cashew juice are shown below:

$$pH = -14468.75723 * A - 2.16790E + 005 * B - 1.24012E + 007 * C + 1.19211 * E + 005 * A * B + 1.32632 * C + 007 * A * C + 1.58973 * C + 007 * B * C, \quad (5)$$

$$\text{Ascorbic acid (mg/100 mL of beverage)} = 0.065310 * A + 0.18573 * B - 1.40277 * C. \quad (6)$$

The effects of tropical fruit juice and water proportions on the pH, ascorbic acid concentration, and acidity of the *Aloe vera*–based beverage are presented in Figure 1. The contour plot of the ascorbic acid concentration versus the proportions of cashew juice (A), water (B), and sweetener (C) in the beverage formulation shows an increasing pH as the proportion of cashew juice increases (Figure 1(a)), which is related to the contribution of organic compounds from the fruit. Likewise, ascorbic acid concentration shows a decreasing trend as the proportion of water in the mixture increases (Figure 1(b)), an effect attributable to the dilution principle, since cashew juice represents the main source of this bioactive compound. On the contrary, formulations with higher juice content exhibited the highest concentrations of ascorbic acid, confirming that the blend design directly impacts the nutritional value of the final product. This behavior is consistent with reports in the literature, which have shown that in blended juice systems, the proportion of the vitamin C source ingredient significantly influences its final concentration. Studies such as those by Theba et al. [37] on functional beet and fruit juices and by Jalil Aripin et al. [38] on beverages made with noni, pineapple, and pitahaya agree that formulations with a higher proportion of juice with higher ascorbic acid content result in higher concentrations of this compound in the finished product. Together, these results confirm that in functional beverage formulations, adjusting the proportions of ingredients directly impacts the nutritional contribution of vitamin C, which should be considered when designing blends aimed at maximizing the nutritional quality of the product.

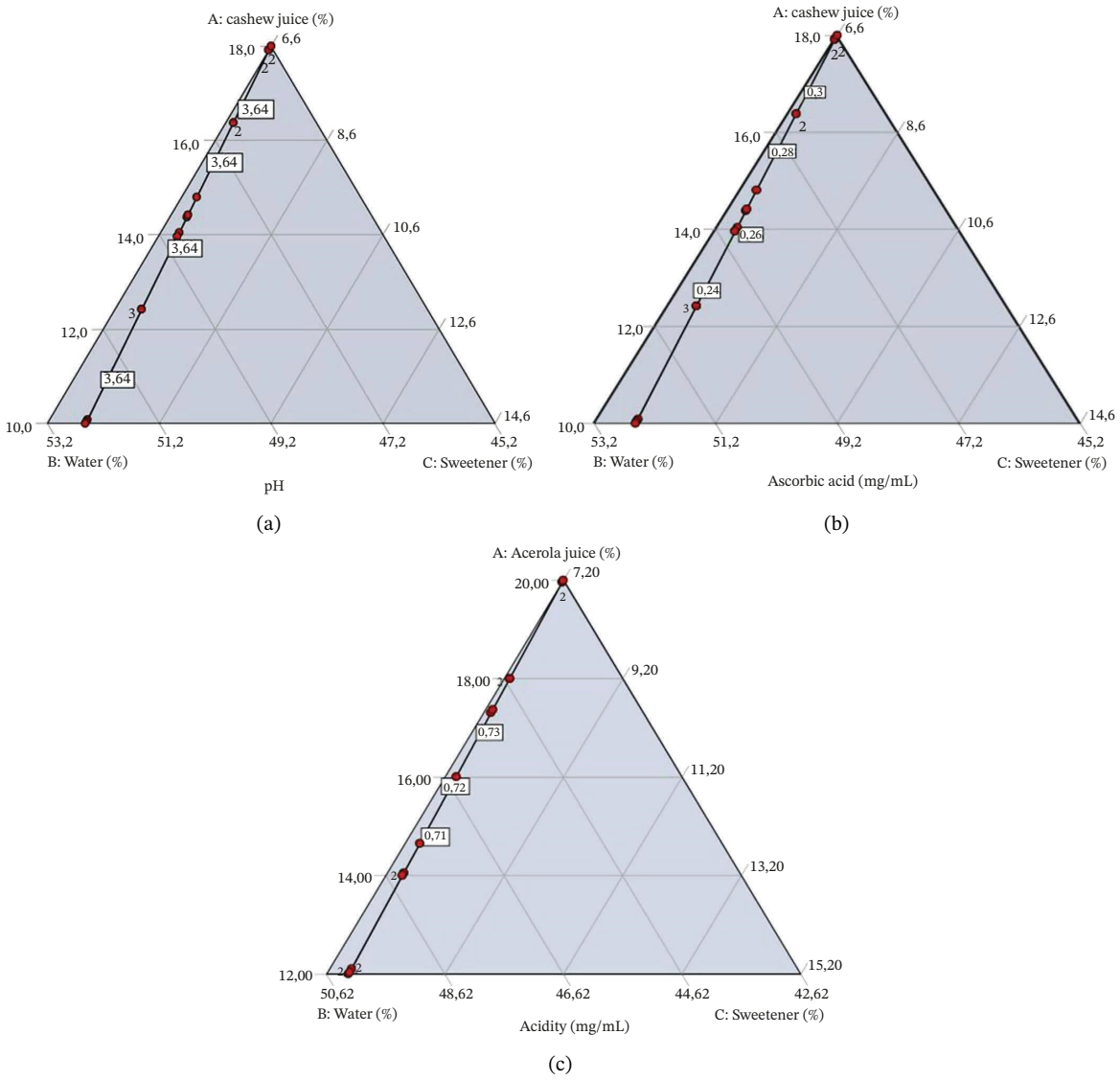


FIGURE 1 | Effect of the ratio of tropical fruit juice to water on the physicochemical properties of the beverage. (a) pH as a function of the proportions of cashew juice, water, and sweetener; (b) ascorbic acid concentration (mg/100 mL of beverage) as influenced by the mixture proportions; (c) titratable acidity (mg/mL) in relation to the ratios of acerola juice, water, and sweetener.

The *Aloe vera* beverages with acerola juice had a pH range of 3.48–3.78, with an average of 3.57 ± 0.07. The soluble solids results showed values between 7.93 and 9.87 with a mean of 8.87 ± 0.46. The acidity ranged from 0.66 to 0.80 mg/mL with a mean of 0.72 ± 0.04 mg/mL. The ascorbic acid content ranged between 25.0 and 58.0 mg/100 mL of beverage with a mean of 0.36 ± 0.08 mg/mL. However, statistically, the variables pH, °Brix, and ascorbic acid did not present significant mathematical models for the results obtained. For its part, acidity showed a significant linear model with an R^2 equal to 52.3%. As the juice concentration increased, an increase in the acidity of the beverage was observed (Figure 1(c)), where the effect of organic compounds on the product is evident. The equation for the pH and ascorbic acid graphs of the acerola juice-enriched beverage is shown below:

$$\text{acidity}(\text{mg ascorbic acid/mL beverage}) = -0.11067 * \text{A} - 0.20831 * \text{B} + 1.64518 * \text{C}. \quad (7)$$

In acerola-based beverages, although the statistical models were less predictive for pH and soluble solids, acidity exhibited a significant linear model ($R^2 = 0.52$), showing a direct dependence on the proportion of acerola juice (Figure 1(c)). This behavior is attributed to the naturally high content of organic acids and vitamin C present in acerola pulp.

These results demonstrate that the mixture design directly controlled the nutritional and physicochemical profile of the

TABLE 3 | Beverage formulation, aloe, and tropical fruit blends.

Formulation	Cashew	Acerola
Fruit	11.224	14.007
Water	51.418	48.329
Sweetener	7.177	7.483
Aloe vera gel	25	
Aloe cubes	5	
Citric acid	0.071	
Na citrate	0.060	
Ca lactate	0.050	
Vit. C	0.001	
Gellan gum	0.028	

beverages, validating the suitability of this approach for functional beverage formulation.

3.3 | Beverage Optimization

The results obtained from the experimental design of the beverages allowed for optimization using response surfaces. Table 3 presents the compositions of the optimized beverages, formulated with tropical fruit juice (cashew and acerola) and *Aloe vera* gel. These formulations were selected for further evaluation in shelf-life studies.

Aloe vera was incorporated into the beverage formulation as a constant functional component. The incorporation of *Aloe vera* gel cubes into beverage formulations is supported by their dual functional role. *Aloe vera* gel contains insoluble polymeric particles and bioactive polysaccharides such as acemannan, which influence the rheological behavior and physical stability of suspensions during processing and storage [12]. Additionally, previous studies have demonstrated that *Aloe vera* gel supplementation increases total phenolic content and contributes to the functional and nutritional enhancement of food systems without compromising sensory properties [39].

The incorporation of *Aloe vera* gel cubes may also influence the physicochemical behavior and physical stability of the beverage matrix. *Aloe vera* gel contains bioactive polysaccharides, particularly acemannan, along with insoluble polymeric particles that have been reported to affect the rheological behavior and stability mechanisms of aqueous suspensions during processing and storage [12]. These polysaccharide-rich structures can exhibit non-Newtonian shear-thinning behavior and modify the apparent viscosity of the continuous phase, potentially contributing to resistance against sedimentation and phase separation phenomena. Similar structural and rheological modifications have been described in food systems enriched with *Aloe vera* gel, where incorporation altered physicochemical and rheological parameters without negatively affecting sensory properties [39]. Therefore, beyond bioactive enrichment, the inclusion of aloe gel particulates in the present formulation may contribute to structural modulation within the beverage matrix.

The concentration and proportion of bioactive compounds in the optimized beverage formulations were determined. Aloin, aloe-emodin, and ascorbic acid were identified and quantified. Accurate determination of aloin in the samples required the development of a calibration curve, which showed linearity with a correlation coefficient of 0.9973. In the final formulated and pasteurized beverages, an aloin content of 0.0012 μg/mL (1.2 μg/L) was detected. This concentration is well below levels of toxicological concern and complies with current regulatory recommendations, which require aloin and other hydroxyanthracene derivatives to be absent or present only at trace levels in food products [14]. No quantifiable traces of aloe-emodin were observed in any of the mixtures analyzed. Figure 2 shows a representative HPLC chromatogram of the clarified *Aloe vera* gel after activated carbon treatment, illustrating the effective removal of aloin prior to beverage formulation. Complete removal of the target compound was achieved, as evidenced by the absence of detectable aloin peaks in the chromatogram shown in Figure 2. These results demonstrate that the concentrations of anthraquinones (aloin and aloe-emodin) present in the beverages are extremely low. In this sense, these levels are unlikely to have a perceptible impact on flavor, suggesting that they do not affect the sensory acceptability of the formulations, in line with the observations by Moselhy et al. [40], who reported that the addition of *Aloe vera* to functional juices did not compromise sensory properties, maintaining high levels of acceptance.

Furthermore, the low aloin content detected in the final beverages (0.0012 μg/mL) is relevant from a toxicological and regulatory perspective, given that at these concentrations, the risk of unwanted side effects, such as the laxative or abortive effects described for higher levels of this substance, is considerably reduced [41]. In this context, it is important to establish rigorous control over aloin levels in commercial products to ensure their safety and meet the required quality standards to avoid potential risks to consumer health [42].

The calibration curve for determining ascorbic acid showed a correlation of 0.997706. Meanwhile, the optimized beverages recorded ascorbic acid contents of approximately 158 mg/100 mL of beverage for both the cashew and acerola formulations.

HPLC analysis confirmed the presence and accurate quantification of ascorbic acid, as well as the effective removal of aloin from the *Aloe vera* gel prior to beverage formulation. The calibration curves showed excellent linearity ($R^2 > 0.997$). The final beverages presented aloin levels of 0.0012 μg/mL, well below toxicological concern thresholds and in compliance with regulatory recommendations for hydroxyanthracene derivatives in food products. The chromatogram of the treated aloe gel (Figure 2) clearly illustrates the removal of aloin through activated carbon treatment.

The optimized beverages reached ascorbic acid contents of approximately 158 mg/100 mL, demonstrating the effectiveness of the mixture strategy in maximizing the concentration of this bioactive compound while simultaneously ensuring product safety.

3.4 | Stability

The stability results for ascorbic acid content (mg/100 mL) during storage are presented in Figure 3. They indicate that ascorbic acid degradation accelerated significantly at 32°C in both formulations. In the case of cashew, the concentration decreased from 158 to 6 mg/100 mL in 60 days (96.2% reduction), while at 4°C the decrease was less pronounced, reaching 20 mg/100 mL (87.3% reduction). Acerola showed even greater thermal sensitivity, with losses of 97.9% at 32 °C and 89.6% at 4°C.

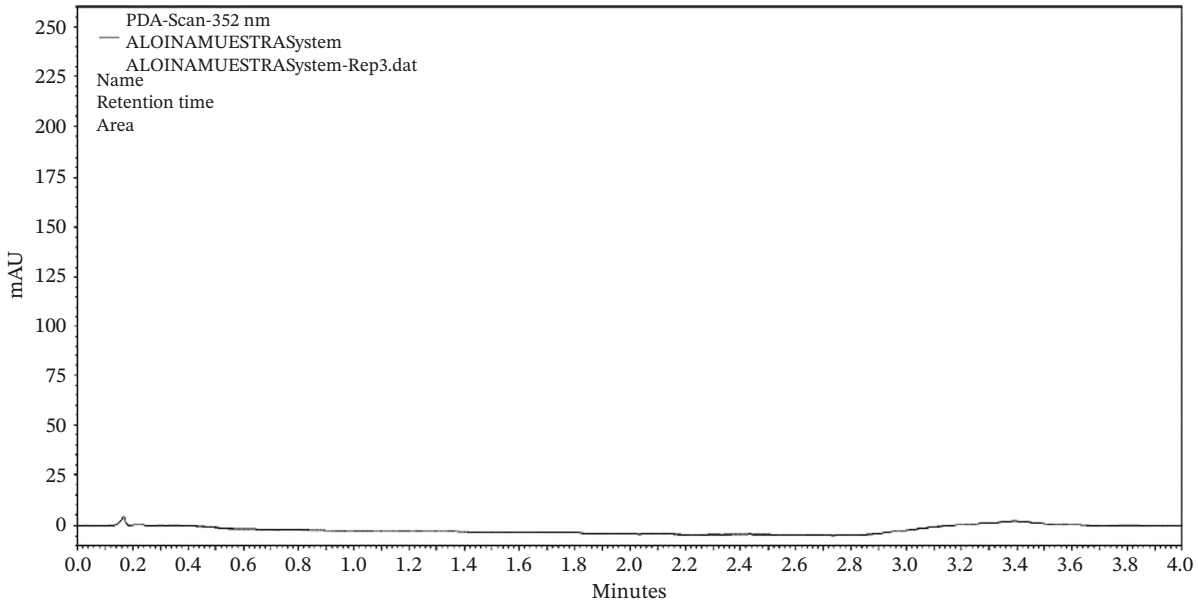


FIGURE 2 | Chromatogram of filtered *Aloe vera* gel after activated carbon treatment, prior to its incorporation into the beverage formulation.

The cashew juice beverage showed significant differences with respect to the temperatures and times tested. In this study, the ascorbic acid content showed significant differences compared to Day 1 at 10, 20, 40, and 60 days at 32°C, while at 4°C, differences were observed at 20, 40, and 60 days. On the other hand, the acerola juice beverage did not show statistically significant differences with respect to the temperatures tested. The ascorbic acid content at 20, 40, and 60 days showed significant differences compared to Day 1 at 32°C, while at 4°C, differences were observed at 40 and 60 days.

These results are consistent with the literature, which has documented that temperature is a critical factor in the stability of bioactive compounds in fruit matrices. The thermal degradation of carotenoids, polyphenols, and vitamin C follows first-order kinetics, with temperature being a determining variable [43, 44]. Additionally, in plant-based beverages, high temperatures accelerate the loss of antioxidants and affect parameters such as pH [45]. This behavior was corroborated in kiwifruit, observing higher rates of vitamin C degradation with increasing temperature [46]. In specific cases of acerola, conventional heat treatments such as ohmic treatments induce the degradation of ascorbic acid according to a first-order model [47]. Even under prolonged freezing conditions, progressive losses have been reported under a similar behavior [48]. These studies support the idea that ascorbic acid exhibits a thermally dependent degradation pattern, which intensifies at elevated temperatures, which is consistent with the results observed in this study.

To describe the degradation behavior of ascorbic acid in beverages during storage at 4°C, the experimental data were fitted to a first-order kinetic model. To do so, the natural logarithm of the relative concentration of vitamin C versus time (t) presented a linear fit consistent with this type of kinetics. The slope of the obtained line ($-k$) was calculated from the experimental data, resulting in −0.0350 d-1, corresponding to the degradation rate

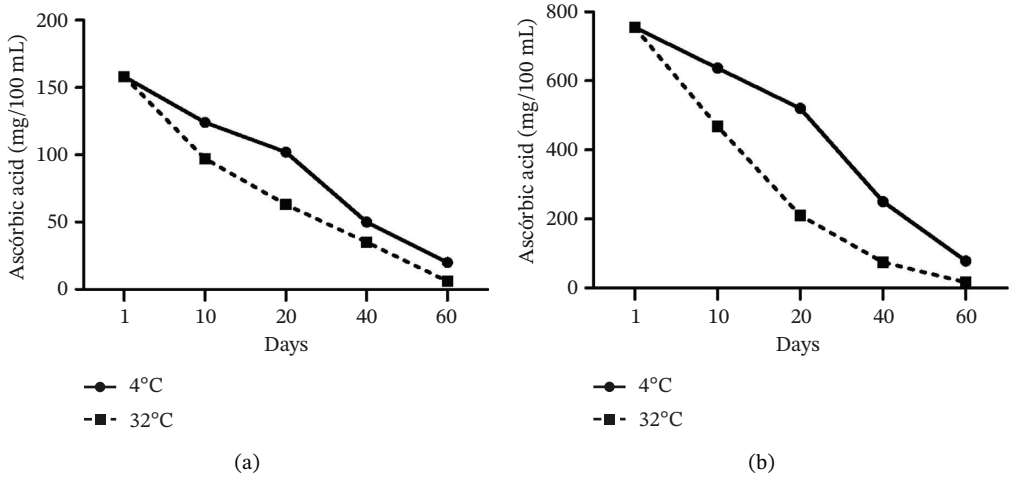


FIGURE 3 | Storage stability of *Aloe vera*–based beverages during 60 days of refrigerated (4°C) or ambient (32°C) storage. (a) Ascorbic acid degradation in beverages formulated with cashew juice; (b) ascorbic acid degradation in beverages formulated with acerola juice.

TABLE 4 | Kinetic parameters for ascorbic acid degradation.

Prototype	T (°C)	Rate constant, k (d^{-1})	E_a (J/mol)
Cashew	25	0.010 ± 0.001 ($R^2 = 0.99$)	40 ± 1 ($R^2 = 0.99$)
	35	0.020 ± 0.001 ($R^2 = 0.99$)	
	45	0.040 ± 0.01 ($R^2 = 0.99$)	
Acerola	25	0.10 ± 0.001 ($R^2 = 0.99$)	50 ± 1 ($R^2 = 0.99$)
	35	0.15 ± 0.004 ($R^2 = 0.99$)	
	45	0.20 ± 0.001 ($R^2 = 0.99$)	

Note: R^2: coefficient of determination of the linear regression.

constant of ascorbic acid at 4°C. This indicates that the apparent degradation occurs at a moderate rate. This result is consistent with reports in the literature, which has documented that in liquid products with acidic pH, vitamin C degradation follows first-order kinetics and can present similar constant values, especially under refrigerated conditions [43, 44, 47].

The observed kinetic fit behavior supports the predictability and temperature-dependent nature of the thermal degradation of ascorbic acid. These results are consistent with reports for other fruit matrices, in which Q_{10} values for fruits and vegetables can vary depending on the food matrix, pH, and storage conditions. In dehydrated matrices such as guavira (*Campomanesia adamantium*) powdered pulp, a Q_{10} of 1.09 has been reported, demonstrating the influence of the low water activity and the physical nature of the matrix on the kinetics of vitamin C degradation [49]. The results confirm that, under refrigeration conditions (4°C), ascorbic acid degradation in beverages occurs at a moderate rate. This suggests that refrigerated storage is adequate for preserving the functional quality of beverages over extended periods.

The degradation of ascorbic acid during storage at 4 °C and 32°C followed a first-order kinetic model (Figure 3). A pronounced temperature effect was observed, with accelerated degradation at 32°C in both beverages. The cashew-based beverages showed a reduction from 158 to 6 mg/100 mL after 60 days at 32°C, whereas under refrigeration the degradation occurred at a slower rate. Acerola-based beverages exhibited even greater thermal sensitivity. The linear relationship obtained by plotting $\ln(X/X_0)$ versus time confirmed the first-order degradation behavior, with a degradation rate constant of $k = 0.035\ d^{-1}$ at 4°C. These findings are consistent with the literature describing vitamin C degradation in fruit beverages as a temperature-dependent first-order process.

3.5 | Shelf Life

To predict the shelf life of the formulated beverages, the ascorbic acid degradation rate constants (k) were estimated at different temperatures. These values allowed the Arrhenius equation to be adjusted and the degradation rate at storage temperature to be determined. The criteria defined were as follows: critical parameter, ascorbic acid (vitamin C); first-order kinetic model; test temperatures, 25°C, 35°C, and 45°C (converted to Kelvin for calculations); shelf-life criterion, ascorbic acid content ≤ 60% of the initial value; initial concentration (C_0), 100 mg/100 mL; and critical concentration (C), 60 mg/100 mL. Table 4 presents the rate constants (k) calculated for each temperature in the beverages made with cashew and acerola.

The Arrhenius equation was linearized to obtain the activation energy (E_a). The values of 1/T (K^{-1}) and $\ln(k)$ were used to calculate the slope of the linear regression, obtaining a value of −5200, from which the resulting E_a is 43.333 J/mol. To predict the shelf life at 25°C (298 K), given that experimentally $k \approx 0.010\ d^{-1}$ at this temperature, this value was directly used to estimate the shelf life using the first-order kinetic equation [$\ln (C_o/C) = k*t$]. The shelf life of the formulated beverages was estimated at approximately 51 days at 25°C, which ensures adequate ascorbic acid content during this period under moderate room temperature storage conditions. This behavior is consistent with the degradation kinetics reported for other fruit beverages, where vitamin C loss typically conforms to first-order models with temperature as the main determining factor. Studies on maqui [26], blood orange [50], and pineapple [51] have shown similar patterns, with activation energies ranging from 14 to 135 kJ/mol. The value obtained in this study (43 kJ/mol) falls within this range, supporting the fact that the kinetics observed in acerola and cashew beverages are consistent with those expected for acidic fruit matrices, where ascorbic acid stability is a critical parameter for defining shelf life.

The degradation rate constants obtained at different temperatures enabled the application of the Arrhenius model to estimate the activation energy ($E_a = 43$ kJ/mol) and to predict the shelf life at 25°C. Using the defined criterion of 60% retention of the initial ascorbic acid content, the shelf life of the beverages was estimated at approximately 51 days. This value is consistent with reports for other acidic fruit beverages and confirms that the optimized formulations maintain their functional quality for extended periods under moderate storage conditions.

4 | Conclusion

Cashew, acerola, and *Aloe vera*–based beverages represent an excellent option for consumers seeking healthy, nutritious, and functional alternatives. This study demonstrates the feasibility of commercializing this beverage, given its high stability, attractive sensory profile, and health benefits. Changes in the temperatures evaluated directly impact shelf life, with greater ascorbic acid degradation occurring at 60°C. The estimated shelf life of the nectar at 25°C is approximately 51 days, based on the degradation of ascorbic acid to 60% of its initial value. The results obtained suggest that this product could have a positive impact on health, particularly in protecting against oxidative stress and supporting the immune system. It also contributes to the comprehensive use of tropical fruits from the Colombian Caribbean region, promoting agroindustrial innovation with a focus on consumer health and well-being.

Author Contributions

Jorge Osorio Martinez: data collection, research execution, methodology development, formal analysis, and writing–original draft preparation. Maria Guerra Solano: supervision, writing–review and editing. Rafael Arango Barbas: supervision, writing–review and editing. Guillermo Arrazola Paternina: conceptualization, resources, funding acquisition, supervision, and review.

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Disclosure

All authors have read and approved the final version of the manuscript, consent to its submission for publication, and agree to be fully accountable for all aspects of the research presented.

Ethics Statement

This study did not involve human participants or animal experiments. Therefore, ethical approval was not required. All procedures were conducted in accordance with institutional and national research guidelines for studies using plant materials.

Consent

This research did not involve human subjects or participants requiring informed consent.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request. These data are not publicly accessible due to privacy restrictions.

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