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Technological and Shelf-Life Impact of Açaí (*Euterpe oleracea*), Beetroot (*Beta vulgaris*), and Hibiscus (*Hibiscus sabdariffa*) Flours in Whey-Based Cocoa Beverages

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ABSTRACT

Whey, a byproduct of cheese production, is characterized by high lactose, protein, and mineral content with a low-fat composition. Due to its abundance of bioactive compounds, it is considered a dairy byproduct of significant biological value. To evaluate the impact of plant-based ingredients on whey cocoa beverages, 10 formulations were developed using açaí, beetroot, and hibiscus flours. Each flour was incorporated independently at concentrations of 0.5%, 1.5%, and 2.5% (w/v). The study employed a factorial design to examine the interactions among flour type, concentration level, and storage duration, providing a comprehensive assessment of their technological effects. The formulations were assessed for pH, syneresis, water-holding capacity (WHC), instrumental color, and microbiological stability over 20 days (Days 1, 5, 10, 15, and 20). On Day 1, incorporating açaí, beetroot, and hibiscus flours reduced pH and syneresis while increasing color parameters (L^*, a^*, b^*, and C^*) and WHC. During storage, formulations containing beetroot flour exhibited a significant decrease in pH. By the end of storage, syneresis increased across all treatments, including the control. These findings underscore the potential of açaí, beetroot, and hibiscus flours to modulate the technological properties of whey-based cocoa beverages, enhancing their potential functionality and value.

1 | Introduction

Cocoa (*Theobroma cacao* L.) is globally recognized as the primary ingredient in chocolate production [1]. In the dairy beverage industry, cocoa is predominantly powdered, with alkalization often applied to enhance its suitability for beverage formulations [2]. Flavored milk chocolates are among the most consumed dairy beverages, primarily due to their appealing taste, aroma, and potential health benefits [3]. These beverages typically contain cocoa powder, sugar, milk, and whey [4, 5].

Despite their popularity, the consumption of chocolate beverages has declined over time [6]. This decline is attributed to concerns over high sugar content and its negative health impacts on consumers [7]. Excessive sugar consumption has been associated with diabetes, obesity, and cardiovascular diseases [8, 9]. To address these issues, sweeteners such as stevia [10, 11], monk fruit extract [12], and xylitol [13] have been explored as sugar substitutes in chocolate milk beverages. However, preferences for sugary foods remain highly dependent on regional, social, and age-related factors [8]. Additionally, incorporating plant-based flours has been

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shown to influence the sensory attributes of cocoa beverages, including appearance, aroma, taste, and texture. For example, beetroot flour contributes a purple hue, whereas hibiscus flour imparts a more pronounced bitter taste. The specific characteristics of each flour play a critical role in the product's overall acceptance and success [13, 14].

Fruits, due to their high concentrations of bioactive compounds such as vitamins and minerals, have been the focus of numerous studies. The addition of fruit-derived flours has been shown to enhance the technological properties of beverages, including increased syneresis, viscosity, and water-holding capacity (WHC). Examples include flours derived from cupuassu [15], carrot [16], and apple [17]. These flours are characterized by their high fiber content, which offers multiple health benefits, including weight management and improved blood glucose and blood pressure control [18].

In Brazil, legislation permits the inclusion of whey in dairy beverage formulations, where it can be used in powdered, concentrated, or liquid forms [18]. Whey is the primary byproduct of cheese manufacturing, with approximately 9 L of whey generated for every kilogram of cheese produced [19]. In 2023, Brazil's milk production was estimated at 34 billion liters, making it the third-largest milk producer globally [20]. However, the high cost of whey-processing equipment makes its use economically unfeasible for many, particularly small- and medium-sized dairy factories. These factories, which account for more than 27% of Brazil's gross domestic product, often discard whey as waste [21–24]. Therefore, the development of value-added whey-based beverages incorporating plant-derived ingredients represents a feasible strategy for small- and medium-scale dairy industries to reduce waste while generating economically viable products. The selected flours differ in fiber composition, organic acids, and phenolic compounds, which are known to influence physicochemical and microbiological stability. The concentration variation was intentionally designed to evaluate dose-dependent technological effects, particularly water-binding capacity and system stability.

In this context, the hypothesis was proposed that incorporating açaí, beetroot, and hibiscus flours at increasing concentrations would differentially modulate the physicochemical, microbiological, and technological stability of whey-based cocoa beverages, due to their distinct bioactive and structural compositions. This study is aimed at evaluating the effects of these flours on the physicochemical and microbiological properties of whey-based cocoa beverages throughout their shelf life. Finally, this study advances current knowledge by comparing the technological effects of multiple plant-based flours at varying concentrations within a single whey-based beverage system, enabling a comprehensive understanding of their individual and dose-dependent impacts on product stability.

2 | Materials and Methods

2.1 | Materials

The whey used in this study was classified as sweet whey, obtained by enzymatic coagulation during coalho cheese production at the Laboratory of Milk and Dairy Products Inspection and Technology (LaITLácteos), and characterized by a near-neutral pH and a higher lactose content than acid whey. The whey was filtered through a microporous fabric to remove large curd particles, and the filtrate was subsequently pasteurized at 65°C for 30 min. After pasteurization, the whey was stored at 4°C ± 2°C under refrigeration until beverage production.

The ingredients included açaí and beetroot flours (Viva Natureza, Bahia, Brazil), xanthan gum (Leve Croc, Paraná, Brazil), xylitol (Natural Vitta, Bahia, Brazil), and 50% cocoa powder (Nestlé Nordeste, Bahia, Brazil), all of which were procured from local natural product stores in Salvador, Bahia, Brazil. Hibiscus flour (Della Terra, São Paulo, Brazil) and caseinate (Growth Suplementos, Santa Catarina, Brazil) were obtained from reputable online suppliers. Although the flours were commercially sourced and manufacturers did not provide detailed granulometric distributions, they were characterized as fine powders suitable for beverage applications. The particle size distribution was not experimentally determined. Particle size is a critical parameter that influences hydration behavior, viscosity, and physical stability, and its potential impact on results should be considered.

2.2 | Beverage Production

The experimental design was factorial, evaluating three plant-derived flours (açaí, beetroot, and hibiscus) at three concentrations (0.5%, 1.5%, and 2.5% w/v) and a control formulation. The flours were assessed independently to determine the individual effects of flour type, concentration, and storage time on the physicochemical and technological properties of the beverages.

The whey-based beverages were prepared following the methodology described by Salgado et al. [13]. All treatments contained xylitol (3.5% w/v), 50% cocoa powder (7.5% w/v), caseinate (2.5% w/v), xanthan gum (0.25% w/v), and varying concentrations of açaí, beetroot, and hibiscus flours, as shown in Table 1. The whey was heated to 35°C, and ingredients were added in proportions to reach 100% of the final volume. The formulations were prepared under good manufacturing practices and homogenized using a food processor (Philips Walita RI7630, 600 W, São Paulo, Brazil).

The addition of caseinate and xanthan gum provided structural stability and prevented phase separation. The formulation was balanced by progressively reducing whey content as flour concentration increased, ensuring total solids remained constant while incorporating fiber-rich ingredients. Thus, the interplay between whey proteins and biopolymers was crucial in defining the gel structure, thereby minimizing syneresis and optimizing WHC.

The final beverages were packaged in sterile 1000-mL glass bottles and stored at 4°C ± 2°C for 20 days (Figure 1). Microbiological analyses were conducted in accordance with Brazilian legislation [25] to ensure the hygienic quality of the final product.

2.3 | pH Analysis

The pH of the whey and whey-based cocoa beverages was measured using a Kasvi precision pH meter (K39-2014B) at 20°C

TABLE 1 | Concentration of ingredients in whey-based cocoa beverages with flour addition.

	Ingredients (%)					
Treatment	**Xylitol**	**Cocoa**	**Caseinate**	**Xanthan gum**	**Flour**	**Whey**
Control	3.5	7.5	2.5	0.25	0.0	86.25
BAF_S	3.5	7.5	2.5	0.25	0.5	85.75
BAF_M	3.5	7.5	2.5	0.25	1.5	84.75
BAF_H	3.5	7.5	2.5	0.25	2.5	83.75
BBF_S	3.5	7.5	2.5	0.25	0.5	85.75
BBF_M	3.5	7.5	2.5	0.25	1.5	84.75
BBF_H	3.5	7.5	2.5	0.25	2.5	83.75
BHF_S	3.5	7.5	2.5	0.25	0.5	85.75
BHF_M	3.5	7.5	2.5	0.25	1.5	84.75
BHF_H	3.5	7.5	2.5	0.25	2.5	83.75

Note: Control, without flour; S, beverage (0.5% flour); M, beverage (1.5% flour); H, beverage (2.5% flour).
Abbreviations: BAF, beverage with açaí flour; BBF, beverage with beetroot flour; BHF, beverage with hibiscus flour.

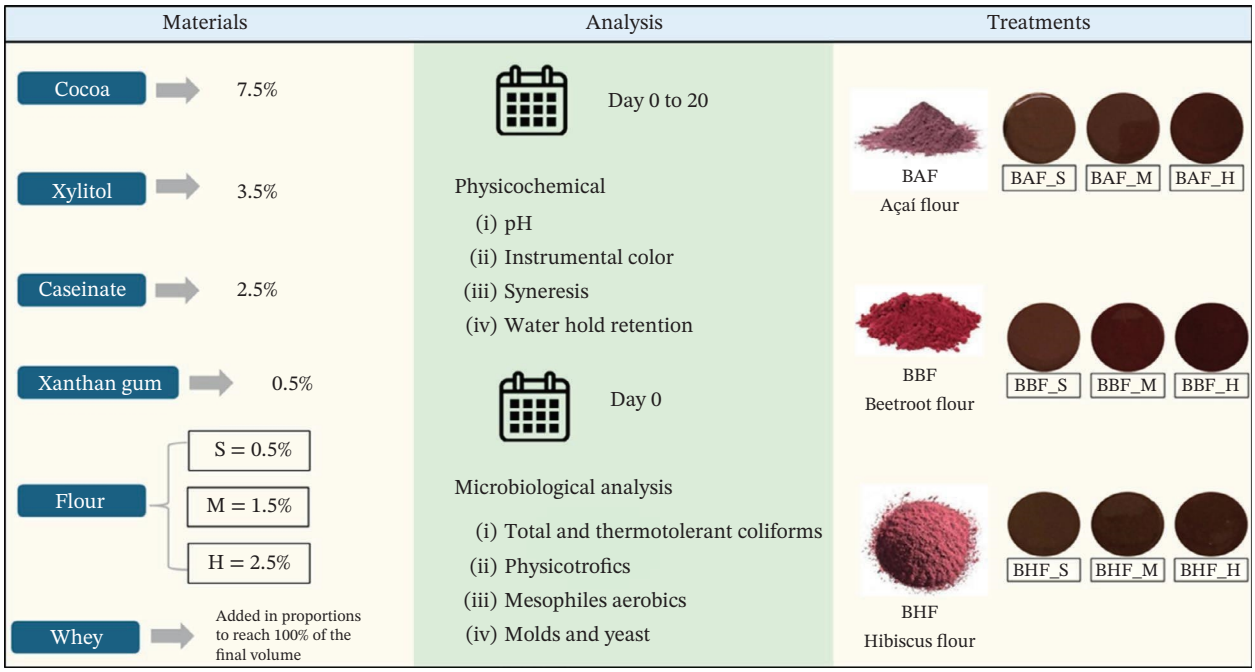

FIGURE 1 | Study design illustrating the stages involved in the experiment of whey-based cocoa beverages. Control, without flour; S, beverage (0.5% flour); M, beverage (1.5% flour); H, beverage (2.5% flour). BAF, beverage with açaí flour; BBF, beverage with beetroot flour; BHF, beverage with hibiscus flour.

over the storage period (Days 1, 5, 10, 15, and 20), as described by dos Santos Rosario et al. [26].

2.4 | Microbiological Analyses

Microbiological quality was assessed on Day 1 for total and thermotolerant coliforms and psychrotrophic bacteria, and throughout storage (Days 1, 5, 10, 15, and 20) for aerobic mesophiles, molds, and yeasts (Figure 2). Samples (25 g) were diluted in 225 mL of 0.1% peptone water (Merck, Darmstadt, Germany) and subjected to serial dilutions. Selective media were used for enumeration: lauryl sulfate tryptose (LST) broth for coliforms (results expressed as MPN mL^{-1}), dichloran rose bengal chloramphenicol (DRBC) agar for molds and yeasts (28°C for 5–7 days), plate count agar (PCA) for aerobic mesophiles (37°C for 24–48 h), and tryptic soy agar (TSA) for psychrotrophic bacteria (4°C ± 2°C for 7–10 days). Enumeration was performed using an electronic counter (Flash & Go, IUL Instruments, Barcelona, Spain), and results were expressed as log CFU mL^{-1}. Analyses adhered to American Public Health Association guidelines [27].

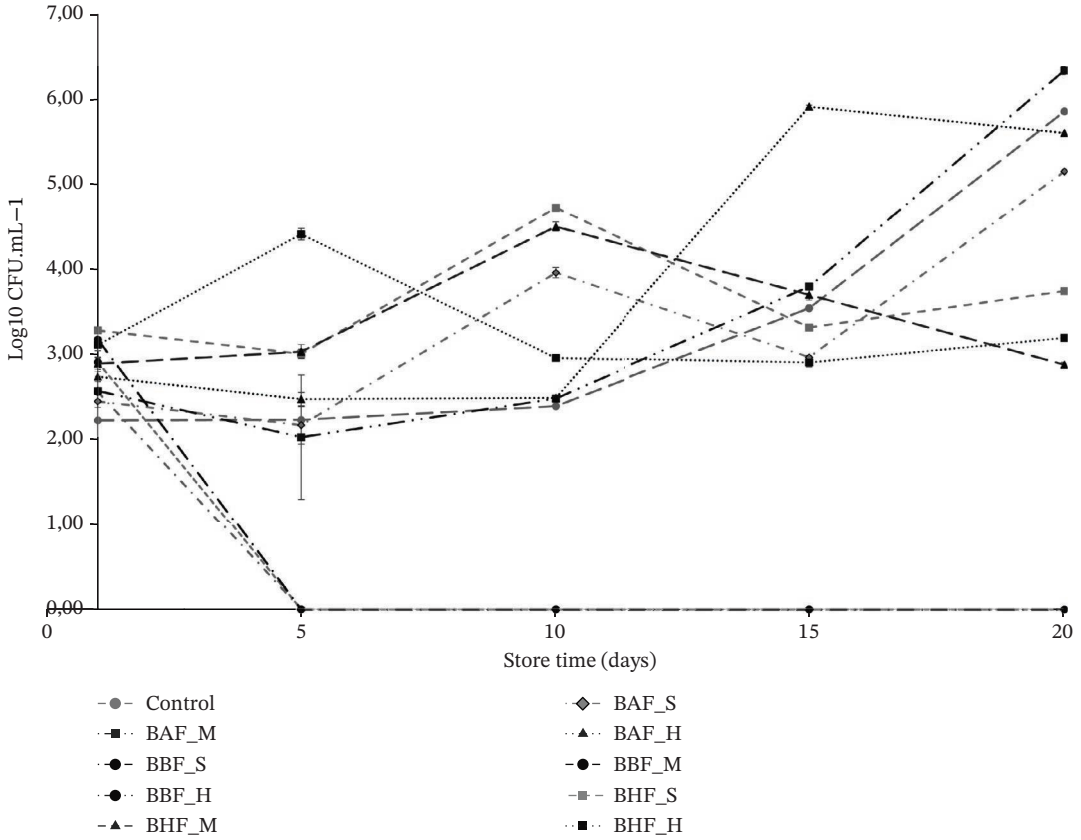

FIGURE 2 | Aerobic mesophiles' count in cocoa whey-based beverages with flour addition as influenced by treatments over 20 days of storage at 4°C. Control, without flour; S, beverage (0.5% flour); M, beverage (1.5% flour); H, beverage (2.5% flour). BAF, beverage with açaí flour; BBF, beverage with beetroot flour; BHF, beverage with hibiscus flour.

2.5 | Instrumental Color Analyses

Color parameters, including L^* (lightness, 0 = black, 100 = white), a^* (+red, −green), b^* (+yellow, −blue), chroma (C^*, color intensity), and hue angle ($h°$), were measured using a Minolta Chroma Meter CR-400 (Minolta Camera Co. Ltd, Osaka, Japan) at each storage interval. The total color difference (ΔE) relative to the control was calculated using Equation (1) [28]:

$$\Delta E = \sqrt{\left(L_n^* - L_1^*\right)^2 + \left(a_n^* - a_1^*\right)^2 + \left(b_n^* - b_1^*\right)^2} \quad (1)$$

2.6 | Syneresis and WHC

Syneresis and WHC were evaluated using the methodology described by Gutiérrez-Álzate et al. [15] with modifications. Ten grams of whey-based cocoa beverages were centrifuged at 3300 rpm for 15 min at 4°C to separate the supernatant and precipitate. Syneresis was calculated as the ratio of the whey weight (supernatant) to the sample weight, whereas WHC was defined as the ratio of dehydrated gel (precipitate) to the sample weight, using Equations (2) and (3), respectively:

$$\text{WHC}\,(\%) = \frac{\text{weight of precipitated (g)}}{\text{weight of samples (g)}} \times 100 \quad (2)$$

$$\text{WHC}\,(\%) = \frac{\text{weight of precipitated (g)}}{\text{weight of samples (g)}} \times 100 \quad (3)$$

2.7 | Statistical Analyses

All the experiments were conducted in three replicates, and the data obtained were presented as means ± standard deviations (SDs). A one-way ANOVA followed by Tukey's test ($p < 0.05$) was applied to the centesimal results. For pH, syneresis, WHC, instrumental color, and microbial data, a two-way ANOVA followed by Tukey's multiple comparison tests ($p < 0.05$) was performed. Analyses were conducted using GraphPad Prism 8 (San Diego, California, United States).

3 | Results and Discussion

3.1 | pH Values of Whey-Based Cocoa Beverage

The variation in pH during storage is presented in Table 2. The addition of plant-based flours significantly decreased the pH of whey-based cocoa beverages ($p < 0.05$) compared to the control treatment. On Day 1, all formulations containing flours exhibited lower pH values than the control, with the magnitude of the reduction depending on both the type and concentration of the flour. These findings align with prior research indicating pH values in chocolate milk beverages ranging from 6.33 to 6.88 [29] as observed in the present study. The flour concentration also influenced pH values, with higher concentrations (BAF_H, BBF_H, and BHF_H) producing greater reductions in pH ($p < 0.05$). Among the treatments, beverages with hibiscus flour resulted in the most pronounced acidification,

TABLE 2 | pH (mean ± SD) of cocoa whey-based beverages with flour addition during 20 days of storage at 4°C.

Attribute	Treatment	Day 1	Day 5	Day 10	Day 15	Day 20
pH	Control	6.88 ± 0.02^{aA}	6.76 ± 0.01^{aB}	6.84 ± 0.00^{aA}	6.88 ± 0.04^{aA}	6.88 ± 0.01^{aA}
	BAF_S	6.81 ± 0.01^{bB}	6.75 ± 0.01^{aC}	6.80 ± 0.01^{cB}	6.86 ± 0.01^{aA}	6.85 ± 0.01^{bA}
	BAF_M	6.78 ± 0.01^{cB}	6.71 ± 0.01^{aC}	6.81 ± 0.01^{bcA}	6.80 ± 0.03^{bAB}	6.79 ± 0.01^{cAB}
	BAF_H	6.77 ± 0.00^{cA}	6.69 ± 0.01^{aC}	6.82 ± 0.01^{bD}	6.76 ± 0.01^{cA}	6.73 ± 0.01^{dB}
	BBF_S	6.50 ± 0.02^{dB}	6.56 ± 0.01^{bA}	6.04 ± 0.00^{eC}	4.54 ± 0.01^{fgD}	4.40 ± 0.01^{hE}
	BBF_M	6.46 ± 0.00^{eA}	6.29 ± 0.03^{cB}	5.76 ± 0.00^{fC}	4.56 ± 0.01^{fD}	4.47 ± 0.01^{iE}
	BBF_H	6.33 ± 0.00^{fA}	6.29 ± 0.01^{cB}	5.43 ± 0.01^{gC}	4.47 ± 0.01^{gD}	4.38 ± 0.00^{jE}
	BHF_S	6.21 ± 0.01^{gA}	6.18 ± 0.02^{dB}	6.22 ± 0.01^{dA}	6.16 ± 0.02^{dB}	5.60 ± 0.01^{eC}
	BHF_M	5.31 ± 0.01^{hAB}	5.32 ± 0.14^{eA}	5.26 ± 0.01^{hAB}	5.21 ± 0.00^{eAB}	5.18 ± 0.01^{fB}
	BHF_H	4.63 ± 0.01^{iB}	4.60 ± 0.00^{fC}	4.56 ± 0.00^{iA}	4.51 ± 0.01^{fgD}	4.52 ± 0.00^{gD}

Note: $^{a–g}$Different lowercase letters in the same column indicate significant differences among treatments of whey-based cocoa beverages ($p < 0.05$). $^{A–E}$Different capital letters in the same lines indicate significant differences among treatments of whey-based cocoa beverages ($p < 0.05$). Control, without flour; S, beverage (0.5% flour); M, beverage (1.5% flour); H, beverage (2.5% flour).

Abbreviations: BAF, beverage with açaí flour; BBF, beverage with beetroot flour; BHF, beverage with hibiscus flour.

followed by beetroot and açaí flours. This behavior can be attributed to the presence of organic acids, particularly ascorbic acid, in hibiscus [30]. A concentration-dependent effect was observed, with higher flour levels resulting in significantly lower pH values ($p < 0.05$), indicating that the addition of these ingredients directly modulates the system's acidity. During storage, pH changes became more pronounced, particularly in BBF_M and BBF_H treatments containing beetroot flour, where significant reductions in pH ($p < 0.05$) were observed by Day 5. Notably, certain formulations, such as BBF_S and BHF_M, demonstrated relative stability in pH, indicating that these concentrations may support better physicochemical stability. However, by Day 20, treatments with beetroot flour exhibited substantial pH declines (e.g., reductions of 31%–32% compared to Day 1), likely due to the degradation of betalains into yellowish betalamic and isobetalamic acids, consistent with findings by Abdo et al. [31]. Similar trends have been reported in yogurt containing beetroot extracts, where lactic acid bacteria activity during fermentation contributed to a decline in pH [32].

Formulations containing beetroot flour exhibited a significant reduction in pH over storage ($p < 0.05$), with a pronounced decline after Day 10, indicating potential limitations for shelf-life stability, as lower pH can adversely affect flavor, texture, and product longevity. This decline may be associated with betalain degradation and the formation of acidic compounds.

In contrast, beverages formulated with açaí flour exhibited relatively stable pH values throughout storage, especially at intermediate concentrations, suggesting a potential buffering effect or interactions with other formulation components. Hibiscus-based formulations maintained consistently low pH values throughout the storage period, with only minor variations, reflecting the ingredient's intrinsic acidity. Overall, these findings demonstrate that both flour type and concentration significantly affect pH behavior, with beetroot flour exerting the greatest impact on pH reduction during storage, which may have implications for product stability and sensory acceptance.

3.2 | Microbiological Analyses

All formulations complied with Brazilian regulatory standards for dairy beverages at the initial stage, as no total or thermotolerant coliforms or psychrotrophic microorganisms were detected. Initial counts of aerobic mesophilic microorganisms were below the acceptable limit (< 5 log CFU mL^{-1}), confirming the adequacy of processing and hygienic conditions. The addition of plant-based flours significantly influenced microbial behavior during storage ($p < 0.05$). Notably, formulations containing beetroot flour exhibited a reduction in aerobic mesophilic counts after Day 5, with some treatments showing undetectable levels. This antimicrobial effect is likely associated with bioactive compounds present in beetroot, such as phenolics and betalains, which have been reported to disrupt microbial metabolism and induce apoptosis-like mechanisms in bacteria [33, 34]. It is important to note that bioactive compounds were not directly quantified in this study, and therefore, functional claims should be interpreted with caution.

This observation can be justified by the decrease in pH in beverage samples containing beetroot flour, which could reduce bacterial counts over time. In contrast, beverages containing hibiscus flour showed relatively stable microbial counts throughout storage, suggesting a moderate inhibitory effect. Meanwhile, control and açaí-based formulations exhibited stable counts during the initial storage period, followed by a significant increase after Day 10 ($p < 0.05$), indicating a gradual loss of microbiological stability.

Molds and yeasts were detected at low levels across treatments, with slightly higher initial counts observed in some hibiscus and beetroot formulations; however, these remained within

TABLE 3 | Color parameters' values (mean ± SD) of cocoa whey-based beverages with flour addition during 20 days of storage at 4°C.

Attribute	Treatment	Day 1	Day 5	Day 10	Day 15	Day 20
L^*	Control	15.41 ± 1.15^{cC}	20.87 ± 1.60^{cB}	25.45 ± 0.71^{abA}	24.72 ± 0.40^{bA}	24.52 ± 0.76^{bA}
	BAF_S	18.21 ± 1.57^{bcC}	24.48 ± 0.58^{aA}	25.28 ± 0.52^{abAB}	27.55 ± 1.92^{aAB}	26.15 ± 0.56^{aA}
	BAF_M	19.27 ± 1.06^{abC}	23.12 ± 0.53^{abB}	24.27 ± 0.49^{bcAB}	25.22 ± 0.48^{bA}	23.81 ± 0.18^{bB}
	BAF_H	18.33 ± 1.39^{bB}	23.82 ± 0.57^{aA}	23.83 ± 0.66^{bcA}	25.21 ± 0.83^{bA}	23.52 ± 0.26^{bA}
	BBF_S	17.94 ± 0.66^{bcC}	23.05 ± 0.96^{bB}	25.65 ± 0.51^{aA}	26.14 ± 0.73^{abA}	24.33 ± 1.31^{bAB}
	BBF_M	20.03 ± 1.04^{abC}	21.06 ± 0.50^{cBC}	23.34 ± 0.23^{bcA}	24.03 ± 0.26^{bA}	21.82 ± 0.26^{bcB}
	BBF_H	17.82 ± 0.31^{bcC}	20.87 ± 1.04^{cB}	20.87 ± 0.39^{cB}	21.93 ± 0.73^{cB}	23.37 ± 0.42^{bcA}
	BHF_S	21.78 ± 1.42^{aB}	25.01 ± 0.34^{aA}	26.09 ± 0.51^{aA}	25.40 ± 0.49^{bA}	26.23 ± 0.69^{aA}
	BHF_M	20.66 ± 1.48^{abC}	23.57 ± 0.18^{abB}	24.50 ± 0.56^{bAB}	25.63 ± 0.38^{abA}	25.39 ± 0.65^{abA}
	BHF_H	19.04 ± 0.96^{abC}	21.88 ± 0.61^{bcB}	23.19 ± 0.06^{dAB}	23.73 ± 0.78^{bcA}	21.68 ± 0.68^{cB}
a^*	Control	5.67 ± 0.19^{cC}	7.52 ± 0.70^{dB}	9.79 ± 0.34^{eA}	10.04 ± 0.43^{dA}	9.66 ± 0.22^{cA}
	BAF_S	7.15 ± 0.46^{bC}	9.99 ± 0.29^{bB}	10.27 ± 0.43^{deAB}	10.64 ± 0.54^{dAB}	11.17 ± 0.33^{bcA}
	BAF_M	8.03 ± 0.41^{bD}	9.94 ± 0.26^{bcC}	10.73 ± 0.26^{dB}	11.90 ± 0.43^{cA}	10.52 ± 0.36^{cBC}
	BAF_H	7.91 ± 0.52^{bC}	10.99 ± 0.22^{bB}	11.63 ± 0.36^{cB}	12.87 ± 0.68^{bcA}	11.26 ± 0.38^{bcB}
	BBF_S	8.27 ± 0.22^{bC}	11.57 ± 0.43^{abB}	13.00 ± 0.24^{bA}	13.46 ± 0.38^{bA}	11.88 ± 0.78^{bcB}
	BBF_M	11.09 ± 1.03^{aC}	12.28 ± 0.77^{aBC}	14.32 ± 0.37^{aA}	15.52 ± 0.51^{aA}	12.66 ± 0.30^{bB}
	BBF_H	10.04 ± 0.63^{aD}	12.69 ± 0.80^{aC}	14.41 ± 0.31^{aB}	15.52 ± 0.82^{aB}	17.71 ± 0.94^{aA}
	BHF_S	7.63 ± 0.19^{bC}	8.72 ± 0.32^{cB}	9.79 ± 0.32^{eA}	9.72 ± 0.29^{dA}	7.79 ± 0.23^{dC}
	BHF_M	7.14 ± 0.64^{bC}	8.44 ± 0.20^{cdB}	9.47 ± 0.36^{eA}	10.28 ± 0.28^{dA}	9.69 ± 0.52^{cA}
	BHF_H	7.45 ± 0.57^{bC}	8.77 ± 0.44^{cB}	9.86 ± 0.58^{deAB}	10.66 ± 0.29^{dA}	9.46 ± 0.35^{cB}
b^*	Control	4.28 ± 0.23^{abC}	5.76 ± 0.34^{bcB}	8.20 ± 0.45^{aA}	8.84 ± 0.52^{aA}	8.21 ± 0.13^{abA}
	BAF_S	4.84 ± 0.38^{abC}	7.69 ± 0.53^{aB}	7.89 ± 0.66^{abAB}	8.35 ± 0.99^{abAB}	9.22 ± 0.25^{aA}
	BAF_M	4.73 ± 0.25^{abD}	6.15 ± 0.22^{bC}	7.01 ± 0.14^{abB}	8.60 ± 0.70^{aA}	7.01 ± 0.34^{bBC}
	BAF_H	3.81 ± 0.03^{bcC}	6.09 ± 0.33^{bB}	6.79 ± 0.25^{abB}	8.15 ± 0.82^{abA}	6.75 ± 0.36^{bcB}
	BBF_S	4.56 ± 0.22^{abC}	7.43 ± 0.26^{abB}	8.55 ± 0.41^{aAB}	9.46 ± 0.49^{aA}	8.00 ± 0.74^{abAB}
	BBF_M	4.26 ± 0.43^{abC}	5.24 ± 0.56^{cC}	6.78 ± 0.37^{bB}	8.31 ± 0.64^{abA}	6.49 ± 0.42^{cB}
	BBF_H	3.04 ± 0.49^{cC}	4.14 ± 0.60^{dC}	6.09 ± 0.14^{bB}	6.85 ± 0.64^{abAB}	7.89 ± 0.81^{abA}
	BHF_S	5.11 ± 0.21^{aC}	6.44 ± 0.64^{bB}	7.99 ± 0.43^{abA}	8.11 ± 0.53^{abA}	4.65 ± 0.62^{dC}
	BHF_M	4.20 ± 0.42^{bD}	5.46 ± 0.48^{bcC}	6.92 ± 0.49^{bB}	8.12 ± 0.38^{abA}	7.30 ± 0.58^{bAB}
	BHF_H	3.83 ± 0.57^{bcB}	4.89 ± 0.70^{cdAB}	6.02 ± 0.69^{bA}	6.82 ± 0.25^{bA}	6.41 ± 0.50^{cA}

(Continues)

acceptable limits. Overall, the results indicate that beetroot flour is more effective at controlling microbes, whereas açaí flour provides limited antimicrobial protection under the conditions evaluated.

3.3 | Instrumental Color Analyses

Color parameter changes (L^*, a^*, b^*, chroma [C^*], hue angle [$h°$], and ΔE) during storage are summarized in Table 3. The addition of plant-based flours significantly affected all color parameters (L^*, a^*, b^*, C^*, and $h°$) of the whey-based cocoa beverages ($p < 0.05$), with distinct patterns observed depending on flour type and concentration.

The L^* value, indicating lightness (100 = white, 0 = black), increased significantly in all treatments during refrigerated storage ($p < 0.05$). On Day 1, the addition of flours elevated L^* values compared to the control ($L^* = 15.41$), which had the lowest lightness due to the 50% cocoa concentration. Treatments with

TABLE 3 | (CONTINUED)

Attribute	Treatment	Day 1	Day 5	Day 10	Day 15	Day 20
C^*	Control	7.10 ± 0.24^{cC}	9.53 ± 0.69^{cB}	12.77 ± 0.55^{bcA}	13.37 ± 0.66^{bcA}	12.68 ± 0.25^{cdA}
	BAF_S	8.71 ± 0.58^{bC}	12.61 ± 0.53^{abB}	12.95 ± 0.74^{bB}	13.53 ± 1.03^{bcB}	14.48 ± 0.41^{bAB}
	BAF_M	9.32 ± 0.46^{abD}	11.69 ± 0.30^{bC}	12.81 ± 0.29^{bcB}	14.68 ± 0.74^{bA}	12.64 ± 0.49^{cdBC}
	BAF_H	8.78 ± 0.48^{bC}	12.57 ± 0.35^{abB}	13.46 ± 0.42^{bB}	15.24 ± 1.00^{bA}	13.07 ± 0.55^{cB}
	BBF_S	9.44 ± 0.26^{abC}	13.75 ± 0.49^{aB}	15.54 ± 0.41^{aA}	16.44 ± 0.57^{abA}	13.58 ± 1.20^{bcB}
	BBF_M	11.88 ± 1.12^{aC}	13.35 ± 0.92^{aBC}	15.85 ± 0.49^{aB}	17.65 ± 0.76^{aA}	14.33 ± 0.48^{bcC}
	BBF_H	10.48 ± 0.74^{abD}	13.35 ± 0.92^{aC}	15.65 ± 0.32^{aB}	16.97 ± 0.98^{abB}	19.34 ± 1.26^{aA}
	BHF_S	9.05 ± 0.40^{abC}	10.84 ± 0.64^{bcB}	12.63 ± 0.51^{bcA}	12.67 ± 0.55^{cA}	9.07 ± 0.49^{eC}
	BHF_M	8.29 ± 0.76^{bcC}	10.06 ± 0.42^{cB}	11.73 ± 0.57^{bcA}	13.06 ± 0.50^{bcA}	12.13 ± 0.76^{cdA}
	BHF_H	8.40 ± 0.71^{bcC}	10.09 ± 0.70^{cB}	11.68 ± 0.63^{cA}	12.65 ± 0.35^{cA}	11.45 ± 0.55^{cdA}
$h°$	Control	36.99 ± 1.43^{aB}	37.23 ± 0.82^{aB}	39.70 ± 1.02^{aA}	41.33 ± 0.50^{aA}	40.36 ± 0.24^{aA}
	BAF_S	34.10 ± 0.71^{bB}	37.58 ± 1.32^{aA}	37.46 ± 1.22^{abA}	38.01 ± 1.97^{bA}	39.52 ± 0.36^{abA}
	BAF_M	30.48 ± 0.79^{cC}	31.73 ± 0.82^{bcBC}	33.19 ± 0.23^{cB}	35.83 ± 1.36^{bcA}	33.67 ± 0.44^{cB}
	BAF_H	25.76 ± 1.30^{dC}	28.98 ± 0.84^{cB}	30.27 ± 0.48^{dAB}	32.27 ± 1.47^{dA}	30.56 ± 0.87^{dAB}
	BBF_S	28.87 ± 1.14^{cdC}	32.73 ± 0.52^{bB}	33.35 ± 0.93^{cAB}	35.04 ± 0.74^{cA}	33.91 ± 0.92^{cAB}
	BBF_M	21.04 ± 0.50^{eD}	23.03 ± 1.00^{dC}	25.31 ± 0.69^{eB}	28.13 ± 1.11^{eA}	26.76 ± 0.82^{eAB}
	BBF_H	16.75 ± 1.69^{fB}	18.03 ± 1.67^{eB}	22.98 ± 0.55^{fA}	23.56 ± 0.90^{fA}	23.96 ± 1.13^{fA}
	BHF_S	34.42 ± 0.72^{abB}	36.39 ± 1.73^{aAB}	39.20 ± 0.71^{abAB}	39.81 ± 1.07^{abA}	30.62 ± 2.35^{dC}
	BHF_M	30.45 ± 0.49^{cC}	32.87 ± 1.71^{bB}	36.12 ± 1.04^{bA}	38.29 ± 0.54^{bA}	36.97 ± 0.76^{bA}
	BHF_H	27.55 ± 1.76^{dB}	28.89 ± 2.57^{cAB}	31.33 ± 1.50^{cdAB}	32.59 ± 0.62^{cdA}	34.01 ± 1.39^{cA}
ΔE	Control	—	5.97 ± 0.82^{bcB}	11.54 ± 1.87^{aA}	11.28 ± 1.09^{aA}	10.72 ± 1.87^{aA}
	BAF_S	—	7.52 ± 1.74^{aA}	10.62 ± 2.94^{abA}	10.62 ± 2.94^{abA}	9.93 ± 1.25^{abA}
	BAF_M	—	4.53 ± 0.59^{bcdB}	6.14 ± 1.47^{bAB}	8.09 ± 1.62^{bA}	5.69 ± 0.88^{cAB}
	BAF_H	—	6.73 ± 1.79^{abA}	7.28 ± 1.40^{abA}	9.55 ± 2.02^{abA}	6.90 ± 1.11^{bcA}
	BBF_S	—	6.73 ± 0.46^{bB}	9.91 ± 0.98^{abAB}	10.88 ± 1.14^{abA}	8.13 ± 2.03^{bAB}
	BBF_M	—	2.33 ± 1.32^{dB}	5.28 ± 1.83^{bAB}	7.25 ± 1.24^{bcA}	3.31 ± 1.62^{cB}
	BBF_H	—	4.34 ± 1.33^{bcdC}	6.17 ± 0.36^{bBC}	7.90 ± 1.33^{bB}	10.66 ± 1.40^{abA}
	BHF_S	—	3.77 ± 1.29^{cA}	5.68 ± 1.10^{bA}	5.21 ± 1.29^{cA}	4.49 ± 1.76^{cA}
	BHF_M	—	$3.48 \pm 1.38d^{cB}$	5.30 ± 1.12^{bAB}	7.10 ± 1.67^{bcA}	6.23 ± 2.02^{bcAB}
	BHF_H	—	3.40 ± 0.74^{cdB}	5.36 ± 1.60^{bAB}	6.45 ± 1.36^{bcA}	4.33 ± 1.39^{cAB}

Note: $^{a–f}$Different lowercase letters in the same column indicate significant differences among treatments of whey-based cocoa beverages ($p < 0.05$). $^{A–D}$Different capital letters in the same lines indicate significant differences among treatments of whey-based cocoa beverages ($p < 0.05$). Control, without flour; S, beverage (0.5% flour); M, beverage (1.5% flour); H, beverage (2.5% flour).

Abbreviations: BAF, beverage with açaí flour; BBF, beverage with beetroot flour; BHF, beverage with hibiscus flour.

hibiscus flour (BHF_S, BHF_M, and BHF_H) exhibited higher L^* values initially, likely due to the pigmentation properties of hibiscus. Over storage, lightness increased across all formulations, with the most notable changes observed in the control sample (L^* increased to 24.52 by Day 20). Treatments with lower flour concentrations, such as BAF_S and BHF_S, maintained higher L^* values throughout storage.

For the a^* parameter, which measures redness (+) and greenness (−), beetroot flour treatments (BBF_S, BBF_M, and BBF_H) showed significantly higher redness (a^*) values compared to other treatments ($p < 0.05$), with a progressive increase during storage, reflecting the stability and intensity of betalain pigments [26]. Redness increased ($p < 0.05$) across all treatments during storage, indicating enhanced color stability. In contrast,

TABLE 4 | Syneresis and water-holding capacity (mean ± SD) of cocoa whey-based beverages with flour addition during 20 days of storage at 4°C.

Attribute	Treatment	Day 1	Day 5	Day 10	Day 15	Day 20
Syneresis	Control	62.49 ± 1.22^{abA}	61.97 ± 1.91^{bA}	61.95 ± 0.28^{cA}	62.17 ± 1.26^{cdA}	64.70 ± 1.12^{cdA}
	BAF_S	65.51 ± 1.24^{abAB}	60.04 ± 1.68^{bB}	60.84 ± 0.63^{cB}	62.61 ± 1.31^{cdAB}	66.59 ± 0.41^{bA}
	BAF_M	61.32 ± 2.05^{bBC}	59.61 ± 0.70^{bC}	61.30 ± 0.22^{cBC}	62.76 ± 0.80^{cdB}	66.81 ± 0.47^{bcA}
	BAF_H	58.95 ± 2.17^{bcC}	56.07 ± 0.68^{cC}	66.94 ± 0.68^{bA}	58.40 ± 0.77^{eC}	63.03 ± 0.95^{dB}
	BBF_S	54.96 ± 1.67^{cB}	48.02 ± 0.85^{dD}	51.25 ± 0.08^{dC}	64.37 ± 0.72^{cA}	64.97 ± 1.03^{edA}
	BBF_M	46.35 ± 1.87^{dC}	31.25 ± 0.64^{eD}	33.81 ± 0.85^{eD}	60.15 ± 0.08^{deB}	66.22 ± 0.95^{cA}
	BBF_H	41.41 ± 2.53^{dB}	29.17 ± 0.29^{eC}	30.90 ± 0.79^{fC}	61.09 ± 0.32^{dA}	64.45 ± 1.12^{cdA}
	BHF_S	70.97 ± 0.72^{aB}	68.44 ± 1.30^{aC}	71.56 ± 0.85^{aB}	73.95 ± 0.64^{aA}	71.49 ± 0.58^{aA}
	BHF_M	67.32 ± 1.46^{abB}	67.94 ± 0.82^{aAB}	70.06 ± 0.06^{aA}	69.10 ± 0.85^{bAB}	68.96 ± 0.39^{bAB}
	BHF_H	64.27 ± 1.42^{abB}	66.91 ± 0.89^{aA}	61.02 ± 0.60^{cC}	67.47 ± 0.62^{bA}	68.31 ± 0.52^{bcA}
WHC	Control	37.51 ± 1.22^{cdA}	38.03 ± 1.91^{dA}	38.05 ± 0.28^{dA}	37.83 ± 1.26^{bcA}	35.30 ± 1.12^{abA}
	BAF_S	34.49 ± 1.24^{dAB}	39.96 ± 1.68^{dA}	39.16 ± 0.63^{dA}	37.39 ± 1.31^{bcAB}	33.41 ± 0.41^{bA}
	BAF_M	38.68 ± 2.05^{cAB}	40.39 ± 0.70^{dA}	38.70 ± 0.22^{dAB}	37.24 ± 0.80^{bcA}	33.19 ± 0.47^{bcC}
	BAF_H	41.05 ± 2.17^{bcA}	43.93 ± 0.68^{cA}	33.06 ± 0.68^{eC}	41.60 ± 0.77^{aA}	36.97 ± 0.95^{aB}
	BBF_S	45.04 ± 1.67^{bC}	51.98 ± 0.85^{bA}	48.75 ± 0.08^{cB}	35.63 ± 0.72^{cD}	35.03 ± 1.03^{abD}
	BBF_M	53.65 ± 1.87^{aB}	68.75 ± 0.64^{aA}	66.19 ± 0.85^{bA}	39.85 ± 0.08^{abC}	33.78 ± 0.95^{bD}
	BBF_H	58.59 ± 2.53^{aB}	70.83 ± 0.29^{aA}	69.10 ± 0.73^{aA}	38.91 ± 0.32^{bC}	35.55 ± 1.12^{abC}
	BHF_S	29.03 ± 0.72^{eB}	31.56 ± 1.30^{eA}	28.44 ± 0.85^{fB}	26.05 ± 0.64^{eC}	28.51 ± 0.58^{dB}
	BHF_M	32.68 ± 1.46^{deA}	32.06 ± 0.82^{eAB}	29.94 ± 0.06^{fB}	30.90 ± 0.85^{dAB}	31.04 ± 0.39^{cAB}
	BHF_H	35.73 ± 1.42^{dB}	33.09 ± 0.89^{eC}	38.98 ± 0.60^{dA}	32.53 ± 0.62^{dC}	31.69 ± 0.52^{bcC}

Note: $^{a–f}$Different lowercase letters in the same column indicate significant differences among treatments of whey-based cocoa beverages ($p < 0.05$). $^{A–D}$Different capital letters in the same lines indicate significant differences among treatments of whey-based cocoa beverages ($p < 0.05$). Control, without flour; S, beverage (0.5% flour); M, beverage (1.5% flour); H, beverage (2.5% flour).

Abbreviations: BAF, beverage with açaí flour; BBF, beverage with beetroot flour; BHF, beverage with hibiscus flour.

treatments with açaí flour showed lower a^* values on Day 5 than on Day 1, suggesting pigment degradation or interactions with other ingredients. The b^* parameter, representing yellowness (+) and blueness (−), increased over time in all treatments. Treatments with high flour concentrations (BAF_H, BBF_H, and BHF_H) initially exhibited higher b^* values, with yellowness consistently dominating over blue in all samples.

Chroma (C^*), a measure of color intensity, was lowest in control treatments and higher in samples containing beetroot and açaí flours. These trends suggest that plant-based flours enhance color vibrancy, with beetroot flour having the most pronounced effect.

Hue angle ($h°$), indicating perceived color, decreased with higher flour concentrations, particularly in beetroot flour treatments, shifting color perception toward red-purple tones. This was consistent with the high betalain content in beetroot. Although hue angles increased over storage, certain formulations, such as BHF_S, deviated from this trend, warranting further investigation.

Total color difference (ΔE) values increased significantly during storage. On Day 5, most treatments had ΔE values > 3, indicating perceptible color changes, with $\Delta E > 6$ observed in high-flour treatments by Day 15, categorizing these samples into distinct color groups [35, 36].

Overall, beetroot flour exerted the greatest influence on color parameters, particularly redness and color intensity, whereas hibiscus and açaí flours contributed to distinct but less intense color modifications.

3.4 | Syneresis and WHC

Syneresis and WHC results (Table 4) reveal an inverse relationship between flour concentration and syneresis. Higher flour concentrations (BAF_H, BBF_H, and BHF_H) significantly reduced syneresis ($p < 0.05$), emphasizing the technological potential of plant-based flours in stabilizing beverages [15]. Beetroot flour, characterized by high fiber and hydrophilic compound content, exhibited the most pronounced reductions in syneresis, consistent with findings by Kohajdová et al. [37]. The WHC results also confirmed that higher flour concentrations improved water retention, with BAF_H, BBF_H, and BHF_H achieving the highest WHC values. Notably, beetroot flour demonstrated

superior performance in reducing syneresis and increasing WHC, especially by Day 5 of storage ($p < 0.05$), likely due to its structural and compositional properties [38].

Control samples showed no significant syneresis changes over time, whereas formulations with plant-based flours exhibited fluctuations. These fluctuations in both syneresis and WHC were observed, particularly after Day 10, suggesting structural rearrangements within the system. Despite these variations, formulations with higher flour concentrations consistently showed better stability than the control. These results highlight the technological potential of fiber-rich plant-based flours, particularly beetroot, in improving the physical stability of whey-based cocoa beverages by reducing phase separation and enhancing water retention.

4 | Conclusion

The incorporation of açaí, beetroot, and hibiscus flours significantly affected the pH and stability of whey-based cocoa beverages. Although hibiscus flour caused the most immediate pH reduction due to its ascorbic acid content, beetroot flour treatments (particularly at 1.5% and 2.5%) exhibited a more pronounced pH decline after 10 days of storage, suggesting potential shelf-life constraints. Conversely, these same beetroot formulations (BBF_M and BBF_H) demonstrated superior performance in stabilizing the matrix, significantly reducing syneresis and increasing WHC. Color parameters were also notably affected, with beetroot's betalain content enhancing redness (a^*). Although açaí flour provided greater overall physicochemical stability, the strategic use of these plant-based ingredients offers a clear pathway to optimize the potential functional and sustainable dairy products, balancing structural integrity with nutritional enrichment. Future studies should include sensory evaluation and particle size characterization to further elucidate the applicability of these formulations in industrial contexts.

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Disclosure

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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