Journal of Food, Nutrition and Diet Science
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Original Research

Study of the physicochemical stability and microstructure of a vegetable-based popsicle

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Abstract: **Aim:** This study aimed to develop two açaí popsicle formulations, one with banana and one without, in order to evaluate the influence of banana addition on the nutritional profile and physical stability of the final product. **Methods:** Two formulations were prepared and subjected to a series of analyses. The experimental approaches included physicochemical stability tests, color and pH measurements and microstructural characterization. Photon microscopy and a Zetasizer apparatus were employed to analyze the microstructural properties, while standard data analysis procedures and statistical methods were used to assess the significance of the observed differences. **Results:** The addition of banana significantly enhanced the melting and flow stability of the popsicles. Despite this positive effect on texture under thermal stress, the addition of banana did not lead to statistically significant improvements in color, pH or other aspects of microstructure. **Conclusions:** The findings indicate that while the addition of banana improves certain physical properties such as melting and flow stability, it is not a critical component for ensuring overall product stability. Both formulations demonstrated adequate stability, supporting the potential for developing plant-based popsicle alternatives with or without banana supplementation. However, Treatment B proved to be superior due to the functional and sensory properties it can add to the formulation.

Keywords: Edible ice cream, Emulsion, Flow, Microstructure

Received: May 9, 2025; Revised: Jul.15, 2025; Accepted: Jul.21, 2025; Published: Jul.30, 2025

DOI: https://doi.org/10.55976//fnds.32025139680-90

1. Introduction

Banana is a widely consumed fruit among Brazilians, exhibiting both a sweet taste and a pleasant aroma. Brazil is one of the world's leading producers of bananas, with over 530 thousand hectares cultivated across the country, resulting in an annual production of over 6 million tons. In addition to this substantial production and appreciation, bananas are also considered functional foods, as they contain bioactive compounds such as phenolics, carotenoids, biogenic amines and phytosterols, which are associated with various health-promoting effects [1-4].

Similarly, the açaí berry, a fruit endemic to Brazil, is nutritionally rich owing to its high nutritional value, energy content and bioactive compounds [5]. Studies reported that it is a food rich in fiber, fatty acids, carbohydrates and proteins and is also a source of vitamin E and B1, iron, minerals and antioxidant compounds. Therefore this fruit, like the banana, can also be considered a functional food due to its properties [6,7].

The combination of these two fruits can result in a functional food that is highly nutritious and has a pleasant taste. An example of a highly consumed product that can contain these two ingredients is the popsicle, a food with various compositions [8], which makes it easily adaptable to consumer diets [9]. This adaptability facilitates the replacement of traditional ingredients such as milk. Thus, broaden the appeal to more people with dietary restrictions, such as those who are lactose intolerant, allergic to cow's milk protein (CMPA), and those who are known as ovo-vegetarians and strict vegetarians [10-12].

The use of plant-based beverages is an increasingly popular option as an alternative to cow's milk in products. Cirilo, Olivieri, and Martins conducted two studies in their work to assert that the addition of these beverages to edible ice creams improves both nutritional aspects and factors such as melting rate, viscosity and acceptability [13].

Thus, this study aimed to produce two formulations of açaí popsicles with added plant protein and xylitol, with and without banana, in order to evaluate the differences in the physical-chemical stability parameters, color, pH and microstructural characterization of the treatments.

2. Materials and methods

Filtered water, soy extract, Dwarf Cavendish banana, açaí pulp, neutral base, emulsifier, soy cream and xylitol were obtained from different local markets. Table 1 shows the proportions of the ingredients used in the treatments with and without banana.

After weighing all the ingredients, the components of the popsicle were homogenized in a blender for 5 minutes. Subsequently, a rapid pasteurization was performed, during this time the temperature of the mixture was raised to 80 °C for 25 seconds and then rapidly cooled it down [14] using a freezer. The emulsion was then transferred to containers and frozen at approximately -18 °C.

Table 1. Formulation of the popsicle with (Treatment A) and without (Treatment B), including the addition of banana (n = 3)

Ingredients	Treatment A Weight (% = g 100 g-1)	Treatment B Weight (% = g 100 g-1)	Brands
Water	40	45	NA
Açaí pulp	15	20	LM
Dwarf Cavendish banana Pulp	15	0	LM
Soy cream	10	10	Batavo®
Xylitol	10	15	NA
Soy extract	8	8	NA
Neutral base	1	1	NA
Emulsifier	1	1	NA
Total	100	100	NA

Note: NA = Not available; LM = Local market

The banana pulp added to the formulation replaces portions of water, açaí pulp and xylitol. This substitution is possible due to the high natural sweetness of the banana, which allows a reduction of sweeteners in the formulation, as well as the dilution of the açaí to accommodate low-harvest periods and consumer preferences regarding its strong flavor [15].

2.1 pH analysis

For the pH analysis of the frozen dessert, measurements were taken in triplicate for the treatments on the first day of freezing and after 4 weeks of production at approximately -18 ºC. The samples were stored in the freezer and taken approximately 1 hour before measurement. The pH meter (Hanna pH21) was calibrated with buffer solutions of 4.01 and 7.01, and the equipment was rinsed with osmotic water after each measurement [16].

2.2 Color analysis

The color analysis of the frozen dessert was conducted on the first day of freezing and after 4 weeks of production. Measurements were taken in triplicate on the treatments stored in the freezer and taken approximately 1 hour before measurement. The colorimeter (Konica Minolta Chroma Meter CR-400/410) was calibrated before each analysis by immersing the light projection tube into the sample and cleaning it with osmotic water [17].

In the color analysis, each coordinate determines a parameter in the color according to the CIELAB system. The L^* parameter quantifies the brightness of the color, with 0 indicating black and 100 indicating white; the a^* coordinate shows whether the color tends to red (positive) or green (negative); and the b^* coordinate indicates whether the color tends to yellow (positive) or blue (negative) [18].

The color difference (ΔE) of the formulations after storage was calculated according to the following equation:

$$\Delta E = \sqrt{(L^*_{final} - L^*_{initial})^2 + (a^*_{final} - a^*_{initial})^2 + (b^*_{final} - b^*_{initial})^2}$$

2.3 Melting test

A 40 g sample of each frozen popsicle treatment was transferred to a steel sieve (with a 2.5 mm mesh) placed over a 600 mL beaker. With a small time interval between samples, photographs of the flow of the samples were taken at time 0 (when transferred from the freezer to the sieve) and subsequently every 5 minutes for 50 minutes [19].

2.4 Rheological flow test

From the frozen treatments, three 40 g samples of each treatment were taken and transferred to a steel sieve (with a 2.5 mm mesh) placed over a pre-weighed 600 mL beaker. In a temperature-controlled room, the weight of the melted material was collected at 5-minute intervals for 50 minutes, starting from the first drop of drainage. The results were then plotted on a graph as a function of time [19].

2.5 Freeze-thaw test

The frozen desert mixture was placed in a hermetically sealed container and subjected to freezing cycles (-18 °C for 24 hours) and thawing cycles (in the refrigerator for 24 hours) for 14 days, with the first day being 24 hours after the production of the treatment. At the end of each cycle, photographic records were made using a cell phone [16].

2.6 Stability

The liquid mixure was placed in a hermetically sealed container and stored under controlled refrigeration at −18 °C and protected from light for 4 weeks, with approximately 40 mL of the sample. Weekly visual analyses were performed with photographic records taken using a cell phone to assess phase separation or other unexpected phenomena [16].

2.7 Microstructural characterization

2.7.1 Particle size

Zetasizer Nano series instruments (NanoZS, Malvern Panalyticas, Worcestershire, UK) were used to measure the mean hydrodynamic diameter (Z-average) and the polydispersity index (PDI), with the laser incidence angle relative to the sample set at 173º. The zeta potential (ZP) was determined through electrophoretic mobility. For the analysis, aliquots of the formulations were diluted (1:800, v/v) in ultrapure water and measured at room temperature. The values obtained from the average of three measurements for each sample were evaluated [20].

2.7.2 Microscopy

The material of the treatment samples was observed using photon microscopy (Olympus DP72). In this test, 3 drops of the mixture were placed on a mircoscope slide using a Pasteur pipette and covered with a coverslip for analysis. The images of the quadrants were taken to visualize the distribution of particles [16].

2.8 Statistical analysis

The tests were performed with at least three replicates for each sample. Statistical analysis was conducted using ANOVA, calculated with MiniTab 16 software and Tukey's test was applied to detect significant differences ($p > 0.05$) between samples. The graphs were generated using OriginPro software.

3. Results and discussion

3.1 pH and color analysis

The treatment samples were frozen for 4 weeks and analyzed for color and potential of hydrogen (pH) at the beginning and end of this period and the results are presented in the tables below. It should be noted that although the samples were stored in a freezer, they were subjected to temperature stress as they had to be thawed for analysis.

Table 2 shows the variations in the L^*, a^*, and b^* coordinates during the storage at low-temperature for treatments A and B. The L^* coordinate decreased in both the banana and non-banana treatments, indicating that the formulations darkened with time. The a^* and b^* parameters increased in both formulations, moving slightly towards red and yellow.

Table 2. Initial and final average color results in the L^*, a^*, and b^* coordinates of açaí popsicle samples with (A) or without (B) added banana

Sample	Time interval	L^*	a^*	b^*	ΔE
Treatment A	Initial	40.33 ± 0.52^{a}	7.11 ± 0.37^{b}	10.45 ± 0.26^{b}	$1.79 \pm 0,69^{a}$
	Final	39.23 ± 0.15^{b}	$7.57 \pm 0.09^{a,b}$	11.69 ± 0.22^{a}	
Treatment B	Initial	38.55 ± 0.22^{b}	7.42 ± 0.01^{b}	9.02 ± 0.29^{c}	3.07 ± 0.44^{a}
	Final	36.12 ± 0.16^{c}	8.14 ± 0.04^{a}	10.87 ± 0.12^{b}	

Note: L^* represents brightness (0 = black, 100 = white); a^* indicates the green (negative values) to red (positive values) axis; b^* indicates the blue (negative values) to yellow (positive values) axis. Means with the same letters in the same column did not show statistical differences ($p > 0.05$). "Initial" corresponds to the first day of storage, and "Final" to the last day.

Table 3. Initial and final average pH results of açaí popsicle samples with (A) or without (B) added banana

Sample	Time interval	pH
Treatment A	Initial	6.08 ± 0.00^{c}
	Final	6.23 ± 0.06^{b}
Treatment B	Initial	6.30 ± 0.03^{b}
	Final	6.46 ± 0.01^{a}

Note: Means with the same letters in the same column did not show statistical differences ($p > 0.05$). "Initial" corresponds to the first day of storage, and "Final" to the last day.

The overall color difference (ΔE*) is a valuable indicator of visual change in foods, with values around 2 generally indicating a perceptible shift in color, while values above 3 suggest the change could be considered unacceptable from a consumer perspective. Values below 3 are generally imperceptible to the human eye, meaning that the product would appear visually stable [21]. In this study, treatment A presented a ΔE* of 1.79 ± 0.69, indicating a minimal and probably imperceptible change in color. On the other hand, treatment B reached a ΔE* of 3.07 ± 0.44, which exceeds the acceptance threshold and suggests a noticeable difference that may affect consumer preference. In comparison, a ΔE* of 2.17 was determined for ice cream [22], positioning treatment A as more visually favorable.

During the same period, the pH value of the samples was also measured in triplicate and the average of the initial and final values was calculated. The results of this analysis are shown in Table 3

The pH indicates whether the solution is neutral, acidic or basic, based on the concentration of H+ ions. Table 3 shows that there was a slight but statistically significant increase in pH values for both treatment A with banana, and treatment B without banana. However, this increase was not sufficient to change the properties of the popsicles from mildly acidic. Comparing the observed pH values with those reported in the literature, they are consistent with those found in similar matrices. For instance, whey protein-based ice popsicles, as well as vegetable and herb-based popsicles were reported to present pH values around 6.2, supporting the classification of the current formulations as mildly acidic. This similarity suggests that the addition of banana did not lead to a significant deviation in acid–base behavior compared to other fruit- or plant-based frozen dessert products, indicating a stable pH profile [23].

3.2 Melting behavior

Approximately 40 g of the treatment with (A) and without (B) added banana were removed from the freezer and placed on a steel mesh to evaluate the flow over 50 minutes at room temperature. Figure 1 compares the behavior of the formulations at 10-minute intervals until the end of the time period..

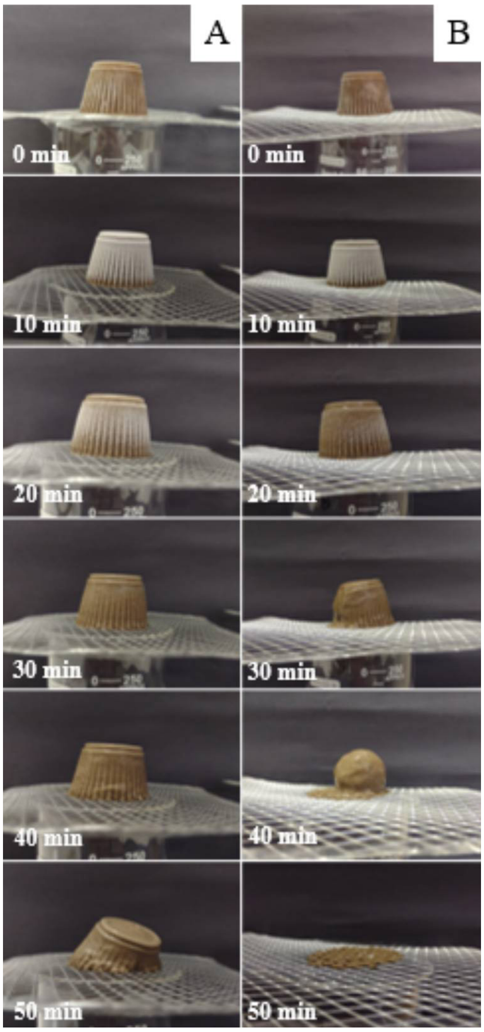

Figure 1. Photograph of the melting test for popsicle treatments with (A) and without (B) added banana.

The popsicle treatments exhibited different resistances and consequently distinct melting times. The sample from treatment B (without banana) began to melt more noticeably between 20 and 30 minutes, defined as the moment when the popsicle starts to lose its initial shape. In contrast, the sample from treatment A (with added banana) only began to melt after 40 minutes. Overall, it can be inferred that formulation A withstands melting slightly longer, while formulation B melts in less than 50 minutes.

The melting phenomenon occurs when the heat transfer penetrates the frozen dessert and causes the ice crystals to melt. This produced water spreads through the microstructure via the unfrozen parts of the matrix, resulting in dripping [24]. Several factors influence this phenomenon, one of which is discussed by Gajo et al., revealing that an increase in the viscosity of the thawed matrix affects the crystallization of the ice and consequently the thawing process [19].

Ferreira explained that viscosity causes the melted water to take longer to flow through the matrix of the frozen desert emulsion during the heat transfer process from the environment to the popsicle [25]. This property is evident in this test, as the more viscous formulation, the one with added banana, exhibited a longer melting time compared to the less viscous formulation without banana.

The added bananas, concurrently, increase the amount of carbohydrates in formulation A, which act as cryoprotectants [26]. This attribute contributes to protection during freezing, which occurs in different ways depending on the agent, one of these is the modification of the formation of ice crystals to prevent excessive growth in certain areas [27]. Thus, it can be inferred that the ice crystals in this formulation were evenly distributed in the popsicle, making the flow through the unfrozen matrix more difficult and reducing the melting rate [24].

3.3 Rheological flow test

Figure 2 presents the flow profiles of the popsicle treatments with and without banana. The graph plots the weight of the melted popsicle material against time, using 40 g samples measured in triplicate. Measurements began with the appearance of the first drop of melt.

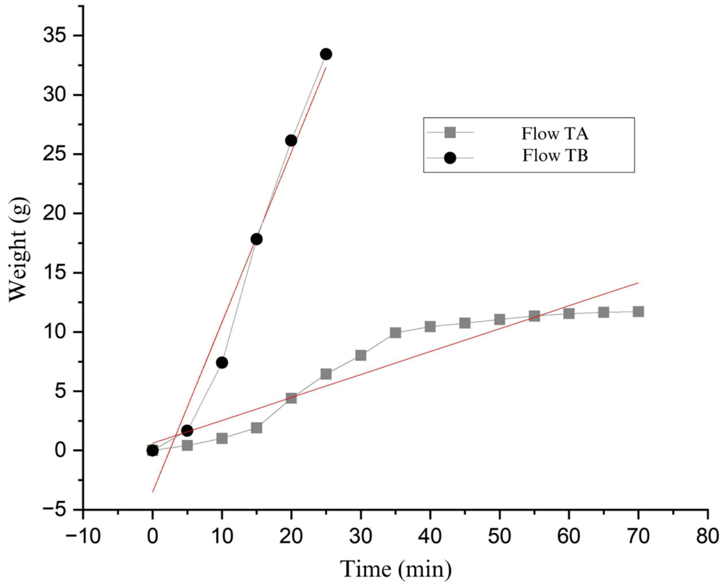

Figure 2. Flow profiles of popsicle treatments with (A/■) and without (B/●) banana.

Table 4. Linear equations, R^2 values, and slopes from the melting test of the popsicles with (A) and without (B) banana added.

Sample	Equation	Melting rate (a)	R^2
Treatment A	y = 0.1934x + 0.6079	0.1934 ± 0.01[b]	0.8897
Treatment B	y = 1.4341x – 3.5152	1.4341 ± 0.12[a]	0.9675

Note: Means with the same letters in the same column did not show statistical differences ($p > 0.05$).

Similar to the previous test, a clear difference in melting can be seen between the two popsicle samples. The popsicle without banana (B/●) took just over 20 minutes to thaw completely after the first drop began to flow. In contrast, the banana-added treatment (A/■) did not even reach half of the initial sample weight in terms of melting, even 70 minutes after the first drop started. From Figure 2, it was possible to obtain a linear equation and then determine the thawing rate based on the slope value.

Treatment A had the linear equation $y = 0.1934x + 0.6079$ ($R^2 = 0.8897$) with a slope of 0.1934, while treatment B had the equation $y = 1.4341x - 3.5152$ ($R^2 = 0.9675$) with a slope of 1.4341. The numerical analysis thus highlights the statistically significant difference in melting rates between the treatments, with treatment B exhibiting a higher melting rate compared to treatment A, corroborating the results observed in the melting analysis.

3.4 Freeze-thaw test

Treatments 1 and 2 were placed in hermetically sealed containers and subjected to temperature stress in 24-hour freeze-thaw cycles to assess the stability of formulation. The samples were observed on the first day after 24 hours in the freezer, and on the last day, the 14th day of the cycle (Figure 3).

At the end of the freeze-thaw cycle, the treatments exhibited the presence of air bubbles. This observation is consistent with a study by Rezende et al. (2021), which investigated emulsions with and without avocado pulp. In that study, the emulsion with pulp also showed an increase in volume and the formation of small bubbles at the end of the freezing and thawing cycle [16]. This was attributed to three potential factors: aerobic fermentation; decomposition of the emulsion due to the coalescence of fat droplets [28] or the anomalous effect of water leading to an increase in particle size, which disrupted the emulsion. The analysis suggests that aerobic fermentation occurred in the formulation, as indicated by the presence of smaller air bubbles. Barcelos et al. (2019), in a study on the quality of ice cream sold in Limoeiro do Norte – Ceará, explained that even though ice cream is stored in a freezer at low temperatures, it remains susceptible to contamination due to its high nutrientional content and abundant water content. The article further illustrated this using Staphylococcus aureus, a microorganism capable of surviving at temperatures below -20 °C and enduring freeze-thaw cycles [29].

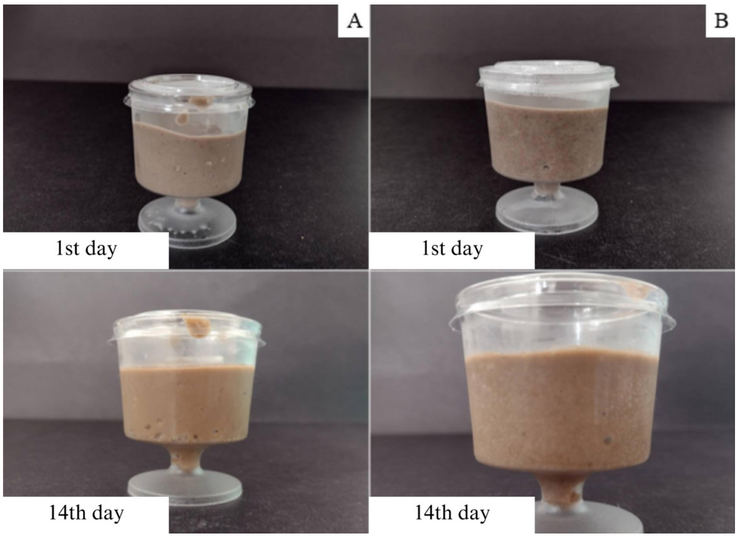

Figure 3. Free-thaw cycles over 14 days for popsicle treatments with (A) and without (B)

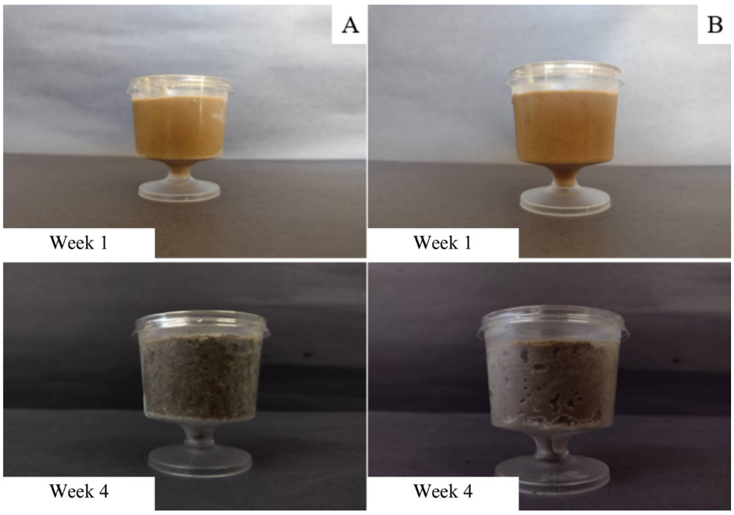

Figure 4. Stability cycle of popsicle treatments with (A) or without (B) banana over 4 weeks

The freeze-thaw test aims to evaluate the stability of the emulsion under temperature stress, bridging the aspects of physicochemical stability, composition and structure. Based on the results, it can be inferred that neither of the two formulations - with or without banana - demonstrated satisfactory physicochemical stability when temperature variations were considered, such as those occurring during the freeze-thaw cycle. The current discussion also encompasses good manufacturing practices, which are not only crucial to consumer health, but are also a legislative requirement. Adhering to these practices will help prevent popsicle contamination [16].

3.5 Stability

Stability was assessed by subjecting a sample of each treatment, with (A) and without (B) banana addition, to a stability cycle in which the samples were stored in a freezer for 4 weeks. Figure 4 compares the samples at the beginning and end of the stability cycle.

In this test, both treatments exhibited numerous air bubbles in the fourth week. In treatment A, more bubbles were concentrated at the top, while in treatment B the bubbles were distributed throughout the container. These bubbles differed from those observed in the freezing and thawing test as they were larger and more numerous. This phenomenon can be attributed to the coalescence of air bubbles, where bubbles collide and merge into larger bubbles, as is a common occurrence in emulsions. Although both formulations contained large air bubbles, the amount in treatment A was lower. One possible explanation for this is that the higher viscosity of treatment A compared to treatment B provided greater resistance, leading to fewer collisions and thus fewer large bubbles. Additionally, as reported by Dominique Langevin, the lifetime of bubbles is also largely determined by their velocity of approach, with coalescence occurring rapidly once contact is made. Higher viscosity impedes this approach, especially in systems where film thinning is already limited, ultimately decreasing the frequency of coalescence events and the formation of larger bubbles [30].

3.6 Microstructural characterization

In the microscopic characterization, the liquid mixture from the treatments with and without banana was deposited onto microscope slides and observed at magnifications of 50 µm and 100 µm. The photomicrographs (Figure 5) illustrate the characteristics of each popsicle matrix before freezing.

From examination of the images, it is evident that air bubbles, identified as lighter spherical structures, and fruit pulp fragments, seen as darker structures, are present. In general, the formulation of treatment B exhibits a higher number of air bubbles, while treatment A shows a greater amount of fruit pulp fragments, which are typical in fruit-flavored products and are considered advantageous for their palatability. This observation was anticipated, given that treatment A includes two fruits, açaí and banana, whereas treatment B contains only açaí.

Air bubbles are an essential part of the matrix in frozen desserts as they are closely related to the perception of creaminess of the product. Smaller bubbles generally enhance this perception. Additionally, air bubbles also play a role in controlling the formation of ice crystals. The dark line observed around the air bubbles represents a lamella formed by proteins and emulsifiers [31].

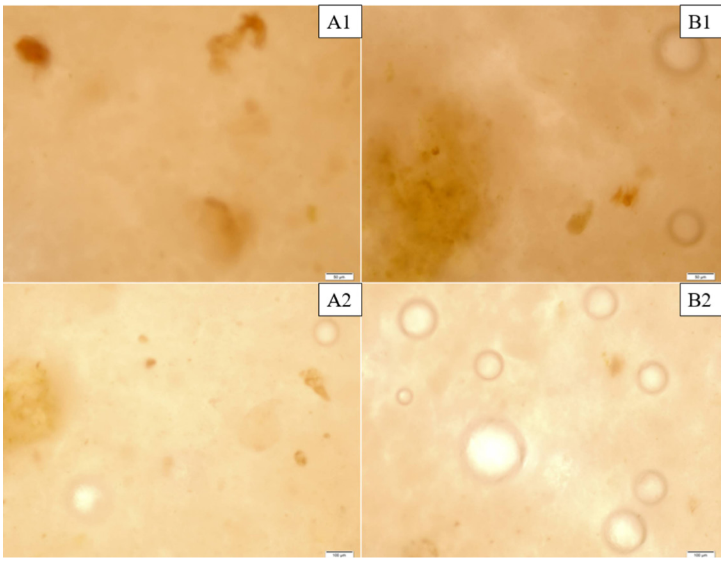

Figure 5. Photomicrographs of the basic açaí popsicle mixture samples with (A1 and A2) and without (B1 and B2) banana: scale bar (A1 and B1) = 50 µm; scale bar (A2 and B2) = 100 µm

Another aspect to discuss regarding the photomicrographs of the formulations is the differences in the sizes of the fruit pulp particles. In the same image, it is possible to observe that the particles are not homogeneous. This characteristic was also evidenced, though in a different manner, with the Zetasizer, which operates at the nanometric level. Table 5 presents the average results with standard deviations from the analysis of the samples.

From Table 5, it is observed that both samples show a Z-average in the micrometric range: 3645 ± 2432 for treatment A and 3971 ± 1348 for treatment B, with a high standard deviation, especially for the formulation containing banana, and no statistically significant difference was observed between the results. Consistently, the samples exhibit PDI values close to 1, with 0.820 ± 0.243 (treatment A) and 0.964 ± 0.099 (treatment B), indicating a significant size variation between the formulations [32], but no statistically significant difference in the parameter when comparing the samples. This is primarily related to the production of the frozen dessert, which was modestly made using a household blender. This situation reflects the conditions in the frozen dessert industry, where there are no high-performance dispersion equipment, such as the Turrax. In such cases, the reduced mechanical shear limits droplet destruction, leading to larger droplet sizes. Generally, higher rotation speeds during emulsification promote greater shear forces, which contribute to forming smaller droplet and improving dispersion [33]

The same was reported by Pena et al. (2021), where a simple setup using the Fisatom equipment was employed to produce a whey-based emulsion, resulting in a significant variability in particle size. The study further adds that the addition of a surfactant to the ingredients or the use of a microfluidizer results in smaller particles [34].

Table 5 also presents the values of the Zeta Potential (ZP). The ZP measures the surface charge of the particles; values close to ±30 mV suggest stability as they reduce aggregation, precipitation or even disintegration of the microemulsion. Sample 1 showed a potential of -33.7 ± 6.13 mV, while sample 2 had a potential of -33.02 ± 1.17 mV. The similar values ($p<0$) suggest that there is no significant difference in stability based on zeta potential; therefore, the addition of banana was not a determining factor in this formulation.

Table 5. Average results and standard deviation of the mean hydrodynamic diameter (Z-average), ZP, and PDI of basic açaí popsicle base samples with or without banana

Treatment	Z-average (nm)	ZP (mV)	PDI
With banana	3645 ± 2432[a]	-33.70 ± 6.13[a]	0,820 ± 0,243[a]
Without banana	3971 ± 1348[a]	-33.02 ± 1.17[a]	0,964 ± 0,099[a]

Means with the same letters in the same column did not show statistical differences ($p > 0.05$).

4. Conclusions

It can be concluded that the addition of banana to the formulation positively influenced the stability in the melting and flow tests. The addition of the fruit increased the viscosity of the formulation of treatment A, thereby reducing the melting speed of the popsicles. However, banana was not a determining factor in the other tests concerning color, pH, stability and freezing-thawing, as both popsicle treatments showed the same trends.

In the physical characterization, air bubbles at 50 and 100 micrometers, as well as fruit pulp fragments (optical microscopy) were observed in the photomicrographs. Additionally, the samples exhibited particle sizes in the micrometer range with a negative charge and significant variability in particle size.

In general, banana was not a decisive factor for the stability of the popsicle, as two stable formulations were successfully produced. However, the study demonstrates that the popsicle is a versatile food that can adapt to different diets and potentially offer health benefits due to ingredients with bioactive properties, such as açaí and banana. Furthermore, the banana-based formulation (Treatment B) is considered the most favorable due to its combination of sensory appeal (particularly natural sweetness), affordability due to the low cost of bananas and the presence of bioactive compounds.

Acknowledgments

The authors would like to thank the Federal University of Juiz de Fora, the Department of Pharmaceutical Sciences, the Graduate Studies and Research Office (PROPP), the Multiuser Laboratory of Bioproducts and Bioprocesses (CENTRALBIO) and the Laboratory for the Development of Nanostructured Systems (LDNano). Special thanks are also extended to Professor Dr. Saulo Marçal Souza for providing access to the equipment used for the microscopic analyses.

Authors' contributions

De Paula Almeida: project administration; conceptualization; methodology; data curation; formal analysis; investigation; writing – original draft; review; submission. Fabiano Freire Costa: conceptualization; project administration; resources; supervision; methodology; writing – review & editing. Felipe Kelmer Müller: data curation; formal analysis; investigation; writing and review; Guilherme Diniz Tavares: writing and review. Nathalia Prado Da Silva: methodology; writing and review.

Conflicting interest

The authors declare no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Availability of data and materials

Data sharing does not apply to this article, as no datasets were created or examined in the course of this study.

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