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*Leucaena Leucocephal*a Gum Exudate: A Novel Natural Stabilizer for Enhancing the Physical Stability of Acidified Milk-Orange Juice Beverages

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Abstract

The growing consumer demand for natural, clean-label food ingredients has intensified the search for novel, sustainable hydrocolloids. This study comprehensively characterizes the exudate gum from *Leucaena leucocephala* and investigates its efficacy as a stabilizer in the technologically challenging system of acidified dairy beverages. Gum was collected from three distinct agro-ecological regions in Zimbabwe, purified via aqueous extraction and ethanol precipitation, and subjected to extensive physicochemical, functional, and rheological analysis. The purified gum was identified as a high-purity (90-94% carbohydrates), acidic (pH 4.5-4.7) polysaccharide with low protein (2.2-3.0%) and fat (<0.4%) content. Fourier-transform infrared (FTIR) spectroscopy confirmed the presence of carboxylate groups and a typical polysaccharide profile. Rheological assessment revealed concentration-dependent viscosity and pronounced shear-thinning behavior, indicative of a structure-breaking network under shear, a desirable property for beverage stabilization. In a milk-orange juice model system, the incorporation of *L. leucocephala* gum at a 0.2% (w/w) concentration resulted in a statistically significant ($p < 0.05$) reduction in sedimentation rate, enhancing physical stability over a 14-day refrigerated storage period compared to the unstabilized control. Critically, sensory evaluation using a 9-point hedonic scale demonstrated that the gum at optimal concentrations (0.1-0.2%) imparted no detrimental effects on color, aroma, taste, or mouthfeel, with overall acceptability scores statistically equivalent to the control. These findings posit *L. leucocephala* gum exudate as a highly promising, natural, and effective stabilizer for clean-label acidified dairy beverages, offering a viable value-added application for a renewable and underutilized biological resource.

Keywords: Acidified Dairy Beverage, Exudate Gum, Leucaena Leucocephala, Natural Hydrocolloid, Rheology, Stabiliser

1. Introduction

The beverage industry is witnessing a surge in demand for innovative products that combine nutritional benefits with sensory appeal. Among these, acidified dairy beverages, such as milk-fruit juice blends, represent a popular category due to their refreshing taste and perceived health benefits [1]. However, their formulation presents a significant technological challenge: the inherent instability caused by the low pH of fruit juice (e.g., orange juice, pH ~3.8-4.0). This acidic environment brings milk casein proteins close to their isoelectric point (pI ~4.6), leading to protein denaturation, aggregation, and ultimately, unsightly sedimentation and phase separation [2]. This defect compromises product quality, shelf-life, and consumer acceptance, necessitating the use of stabilizers.

Stabilizers, primarily hydrocolloids like pectin, gum arabic, and carboxymethyl cellulose (CMC), are employed to mitigate this instability. They function by increasing the viscosity of the continuous phase, thereby reducing the settling velocity of particles (Stokes' law), and/or through electrostatic interactions that prevent protein aggregation [3, 4]. While effective, the current trend in the food industry is shifting dramatically towards "clean-label" products. Consumers increasingly prefer natural, plant-based, and minimally processed ingredients, often eschewing those perceived as synthetic or heavily modified, such as CMC [5]. This paradigm shift has spurred intensive research into discovering and characterizing novel, natural hydrocolloids.

Plant exudate gums, such as gum arabic (*Acacia senegal*), gum tragacanth, and karaya gum, have been used for centuries as natural thickeners, emulsifiers, and stabilizers [6]. These gums are complex polysaccharides secreted by plants as a defense mechanism against injury or stress. Their unique functional properties, often derived from a protein-poly-saccharide complex, make them particularly valuable in food systems [7]. However, reliance on a limited number of exudate gums creates supply chain vulnerabilities and price fluctuations, highlighting the need to explore alternative, underutilized sources.

Leucaena leucocephala (Lam.) de Wit, commonly known as the lead tree or white popinac, is a fast-growing, leguminous tree native to Central America but naturalized in many tropical and subtropical regions, including parts of Africa and Asia [8]. It is known for its drought resistance and ability to thrive in marginal soils. While primarily used for fodder, firewood, and soil improvement, it also produces a gum exudate. Preliminary investigations suggest this gum is a complex, acidic polysaccharide containing galactose, arabinose, rhamnose, and glucuronic acid [9, 10], but a comprehensive characterization of its functional properties and its application in a complex food matrix remains largely unexplored.

Therefore, this study hypothesizes that the exudate gum from *Leucaena leucocephala* can function as an effective natural stabilizer in acidified milk-orange juice beverages by reducing sedimentation without imparting negative sensory attributes. The specific objectives were to: (1) extract and purify *L. leucocephala* gum from different geographical locations in Zimbabwe; (2) characterize its physicochemical, functional, and rheological properties; (3) evaluate its efficacy in minimizing sedimentation and enhancing shelf-life stability in a milk-orange juice model system; and (4) assess its impact on the sensory profile of the final product.

2. Materials and Methods

2.1 Materials

Gum exudates were manually harvested from *Leucaena leucocephala* trees in three distinct regions of Zimbabwe: Zvimba rural area (high rainfall), Harare Belvedere West (medium rainfall), and Bulawayo Burnside (low rainfall), to account for potential agro-ecological variations. Crude gum samples were sorted to remove bark, soil, and other physical impurities. All chemicals used, including absolute ethanol, hexane, sulfuric acid, and sodium hydroxide, were of analytical grade (Sigma-Aldrich, USA). For the beverage formulation, fresh skim cow's milk was obtained from a local dairy, frozen orange juice concentrate (65° Brix), commercial white sugar, food-grade citric acid, and natural orange flavor were used.

2.2 Gum Extraction and Purification

The extraction and purification process followed a modified method from [11] with optimizations. The sorted crude gum was dissolved in distilled water at a ratio of 1:10 (w/v) and hydrated for 24 hours at room temperature (25 ± 2°C) under constant stirring. The resulting viscous solution was first filtered through a muslin cloth to remove large insoluble particles and then through a 0.710 mm sieve. The filtrate was centrifuged (Eppendorf 5804 R, Germany) at 1000 × g for 10 minutes to remove any remaining fine insoluble matter. The clear supernatant was collected, and the gum was precipitated by adding absolute ethanol in a 1:2 (supernatant: ethanol) ratio. The precipitated gum strands were collected, rinsed with fresh ethanol, and dried in a hot air oven (Memmert, Germany) at 40°C for 24 hours. The dried gum was ground using a pestle and mortar, passed through a 0.710 mm sieve to obtain a fine powder, and stored in airtight containers until further analysis. The percentage yield was calculated using the formula (Equation 1):

$$\% \text{ Yield} = (\text{Weight of purified gum} / \text{Weight of crude gum}) \times 100 \quad (1)$$

2.3 Physicochemical Characterization

The color, odor, and texture of the purified gum powder were described sensorily. The pH of a 1% (w/v) aqueous gum solution was measured at 25°C using a calibrated digital pH meter (Mettler-Toledo, USA). Proximate composition was determined using standard AOAC methods [12]. Moisture content was determined by drying 2g of sample in a moisture analyzer (AND, Germany) at 160°C for 8 minutes. Ash content was determined by incinerating 2g of sample in a muffle furnace at 550°C for 8 hours. Fat content was determined by Soxhlet extraction with hexane for 4 hours. Protein content was determined using the micro-Kjeldahl method (Kjeltec 8400, Foss, Denmark), with a nitrogen conversion factor of 6.25. Total carbohydrate content was calculated by difference (Equation 2):

$$100\% - (\%\text{Moisture} + \%\text{Ash} + \%\text{Protein} + \%\text{Fat}). \quad (2)$$

FTIR spectroscopy was performed on a PerkinElmer Spectrum Two spectrometer (USA). The gum powder was mixed with spectroscopic-grade KBr and pressed into a pellet. Spectra were recorded in the range of 4000–500 cm^{-1} at a resolution of 4 cm^{-1}.

2.4 Functional Properties

Water Holding Capacity (WHC) and *Oil Holding Capacity (OHC)* were determined in triplicate according to the method of [13]. Briefly, 0.2 g of gum powder (W_1) was mixed with 10 mL of distilled water or refined cooking oil in a pre-weighed centrifuge tube. The mixture was vortexed for 1 min, allowed to stand for 30 min, and then centrifuged at 2200 × g for 10 min. The supernatant was carefully decanted, and the tube was weighed again (W_2). WHC and OHC were expressed as grams of water or oil held per gram of sample (Equation 3).

$$\text{WHC or OHC (g/g)} = (W_2 - W_1) / W_1 \quad (3)$$

Emulsion capacity was assessed using the method of [14]. Briefly, O/W emulsions with an oil volume fraction of 16.7% were prepared with 0.2-0.4% gum using a high-speed blender. The emulsions were centrifuged at 1300 × g for 5 min, and the volume of the cream layer was measured. Emulsion stability was calculated as:

$$\textbf{\textit{Emulsion Stability (\%) = (Volume of cream layer / Total volume of emulsion) × 100}}$$

2.5 Rheological Characterization

The apparent viscosity of gum solutions (3% w/v) was measured at different temperatures (24, 40, 50°C) using a rotational viscometer (Brookfield DV-II+ Pro, USA) with an

appropriate spindle (LV-3). Flow behavior was characterized by measuring viscosity across a shear rate range of 1-100 s^{-1} at 25°C for solutions of varying concentration (1, 3, 6% w/v). The data was fitted to the Power-Law (Ostwald-de Waele) model (Equation 4):

$$\tau = K\gamma^n, \quad (4)$$

Where τ is the shear stress (Pa), γ is the shear rate (s^{-1}), K is the consistency coefficient ($Pa \cdot s^n$), and n is the flow behavior index (dimensionless).

2.6 Beverage Preparation and Stabilization

A model milk-orange juice beverage was formulated. Skim milk was standardized to 3.0% fat. The standardized milk was pre-heated to 40°C to aid hydration. Citric acid (0.4%), sugar (19%), and the stabilizer (*L. leucocephala* gum at 0%, 0.1%, 0.2%, or 0.5% w/w) were dry-blended and slowly added to the pre-heated milk under constant agitation to ensure complete dissolution. The mixture was homogenized at 250 psi (APV-2000, Denmark), pasteurized at 70°C for 30 min (LTLT), and rapidly cooled to 5°C in an ice bath. The finished beverage was aseptically bottled in 250 mL clear glass bottles and stored at 4°C for the duration of the study.

2.7 Stability and Shelf-life Analysis

Sedimentation was monitored over 14 days. Bottles were stored undisturbed at 4°C. The height of the clear serum layer formed at the top was measured daily using a calibrated ruler, and the sediment volume at the bottom was measured using the "drop height" method [15]. *pH stability* was tracked by measuring the pH of the beverages (Day 1, 10, 20, and 30) using a calibrated pH meter.

2.8 Sensory Evaluation

Sensory analysis was conducted with approval from the departmental ethics committee. A panel of ten semi-trained assessors (5 internal staff familiar with the product and 5 final-year Food Technology students) was selected. Samples (coded with 3-digit random numbers) were presented in a randomized order at 10°C in clear plastic cups. Panelists evaluated the beverages for color, aroma, taste, and texture using a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely). Water and unsalted crackers were provided for palate cleansing between samples.

2.9 Statistical Analysis

All experiments were conducted in triplicate. Data were subjected to one-way and two-way Analysis of Variance (ANOVA) using Minitab 21 software (Minitab Inc., USA). Tukey's Honestly Significant Difference (HSD) test was used for post-hoc analysis to identify significant differences between means at a 95% confidence level ($p < 0.05$). Data are presented as mean ± standard deviation.

3. Results and Discussion

3.1 Gum Yield and Physicochemical Properties

The extraction yield of purified gum was high and consistent across the different geographical sources: Harare (83.61%), Zvimba (80.18%), and Bulawayo (79.85%). These high yields suggest that *L. leucocephala* is a prolific gum producer and that the simple water extraction and ethanol precipitation method is highly efficient for its recovery, making it economically viable for potential scale-up.

The purified gum from all three sources was off-white, odorless, and tasteless, with a fine to smoothly fine powder texture. These are desirable attributes for a food ingredient as they minimize the risk of imparting undesirable colors or flavors to the final product. The pH of 1% aqueous solutions was acidic, ranging from 4.50 (Zvimba) to 4.66 (Harare) (Table 1). This inherent acidity is advantageous for its intended application in acidified beverages, as it would not necessitate significant pH adjustment of the system.

Table 1: Physicochemical properties of L. leucocephala gum from different regions

Property	Harare	Zvimba	Bulawayo
Color	Off-white	Off-white	Off-white
Odor	Odorless	Odorless	Odorless
Texture	Smoothly fine	Smoothly fine	Fine
pH (1% sol.)	4.66 ± 0.02^a	4.50 ± 0.03^b	4.57 ± 0.02^c
Extraction Yield %	83.61 ± 1.2^a	80.18 ± 0.9^b	79.85 ± 1.5^b
Means in the same row with different superscript letters are significantly different ($p < 0.05$).			

3.2 Proximate Composition and Structural Analysis

The proximate composition (Table 2) revealed that the gum is primarily composed of carbohydrates (91.21 - 94.02%), confirming its classification as a polysaccharide. The protein content, though low (2.2 - 3.0%), is critical. In renowned exudate gums like gum arabic, a small protein fraction embedded within a polysaccharide matrix is responsible for its excellent emulsifying properties by facilitating adsorption at the oil-water interface [7]. The variation in protein content (Harare > Zvimba > Bulawayo) showed a positive correlation with the annual rainfall of the collection regions, suggesting environmental factors influence gum composition. The fat content was negligible (<0.4%), and the ash content was very low (0.03-0.06%), indicating high mineral purity. The moisture content ranged from 3.54% to 5.34%, which is well below the 15% threshold that promotes microbial spoilage, ensuring good storage stability [16].

Table 2: Proximate composition (% dry weight basis) of *L. leucocephala gum*

Component	Harare	Zvimba	Bulawayo
Moisture	5.34 ± 0.12^a	4.71 ± 0.18^b	3.54 ± 0.22^c
Protein	3.00 ± 0.10^a	2.60 ± 0.03^b	2.20 ± 0.20^c
Fat	0.23 ± 0.11	0.32 ± 0.02	0.23 ± 0.02
Ash	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
Carbohydrates	91.21 ± 0.28^a	92.33 ± 0.19^b	94.02 ± 0.01^c
Means in the same row with different superscript letters are significantly different ($p < 0.05$).			

The FTIR spectrum (Fig 1) provided insights into the functional groups present. A broad, intense band at around 3280 cm^{-1} is characteristic of O-H stretching vibrations from hydroxyl groups, typical of polysaccharides. The weak bands at around 2920 cm^{-1} are attributed to C-H stretching. The strong bands observed between 1600 cm^{-1} and 1410 cm^{-1} are indicative of asymmetrical and symmetrical stretching vibrations of ionized carboxylate groups (COO^-), confirming the presence of uronic acids (e.g., glucuronic acid) within the polysaccharide structure [17]. This anionic character is crucial as it allows for potential electrostatic interactions with positively charged patches on milk proteins near their isoelectric point. The fingerprint region (1200-900 cm^{-1}) showed complex bands associated with C-O-C and C-

O glycosidic linkages and ring vibrations, confirming the polysaccharide nature of the gum.

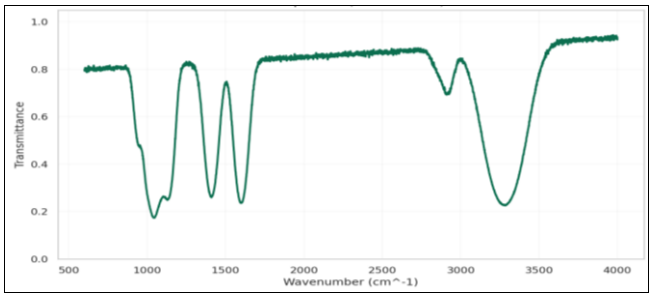

Fig 1: FTIR spectra of purified L. leucocephala gum exudate from Harare, showing characteristic polysaccharide peaks

3.3 Functional and Rheological Properties

The gum exhibited high water solubility in hot water and swelled to form a gel in cold water, dissolving upon vigorous agitation. It was insoluble in organic solvents like ethanol, acetone, and isopropanol, a property exploited for its purification. The WHC and OHC values are shown in Table 3. The WHC is a key functional property, indicating the gum's ability to bind water and increase viscosity, which is directly related to its stabilizing power. The OHC suggests a potential role in stabilizing fatty components in food systems.

Table 3: Functional properties of L. leucocephala gum

Property	Harare	Zvimba	Bulawayo
WHC (g water/g gum)	8.5 ± 0.3^{a}	7.9 ± 0.2^{b}	7.2 ± 0.4^{c}
OHC (g oil/g gum)	3.2 ± 0.1	3.0 ± 0.2	2.8 ± 0.1
Emulsion Capacity (%)	58.45 ± 1.5^{a}	42.28 ± 2.1^{b}	38.44 ± 1.8^{c}

The rheological properties are paramount for a stabilizer. The apparent viscosity of a 3% gum solution decreased with increasing temperature (from 180.0 cP at 24°C to 158.4 cP at 50°C for Harare gum), a behavior typical of hydrocolloids due to the reduction in the stability of hydrogen bonding and increased molecular mobility at higher temperatures [18]. Fig 2 shows that the viscosity increased exponentially with increasing concentration, as expected. More importantly, the flow curves (Fig 3) revealed that all gum solutions exhibited shear-thinning (pseudoplastic) behavior, where viscosity decreases with increasing shear rate. This non-Newtonian behavior is highly desirable for food applications: it provides high viscosity at low shear (during storage, preventing sedimentation) and low viscosity at high shear (during pouring and drinking, ensuring good mouthfeel) [19]. The Power-Law model fitted the data well ($R^2 > 0.98$), with the flow behavior index (n) values significantly less than 1, confirming shear-thinning.

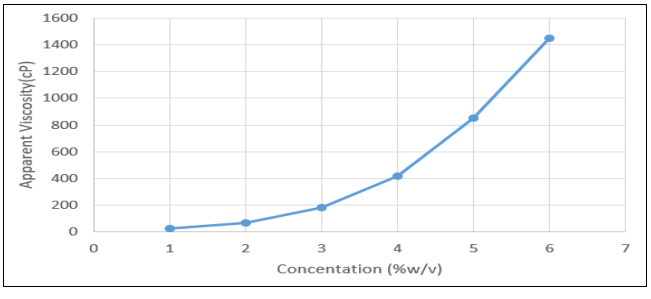

Fig 2: Effect of concentration on the apparent viscosity of L. leucocephala gum (Harare source) solutions at 25°C

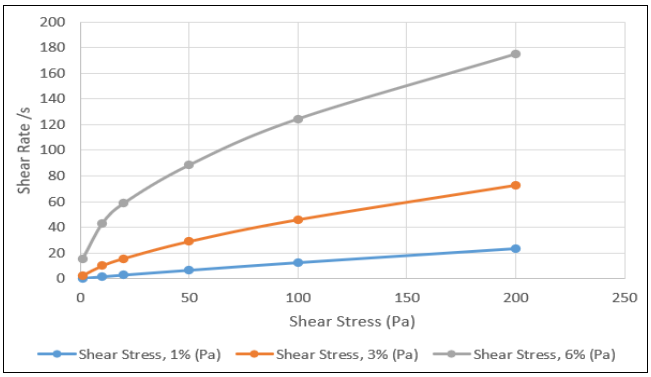

Fig 3: Flow curves (shear stress vs. shear rate) for L. leucocephala gum solutions at different concentrations, demonstrating shear-thinning behavior

3.4 Performance in Milk-Orange Juice Beverage

The primary challenge in milk-juice beverages is physical instability. There was a clear visual difference between the unstabilized control and the gum-stabilized samples after 14 days of storage. The control exhibited significant serum separation and a dense sediment layer. In contrast, samples with 0.2% and 0.5% gum remained homogenous with minimal separation.

Quantitative analysis of sedimentation (Fig 4) showed that no sample exhibited measurable sedimentation for the first 5 days. From day 6 onwards, sedimentation commenced in all samples but progressed at a markedly slower rate in the gum-containing beverages. By day 14, the sediment volume in the control sample was significantly higher ($p < 0.05$) than in all treated samples. The sample stabilized with 0.2% *L. leucocephala* gum showed a 5.94% reduction in sedimentation compared to the control, demonstrating a significant enhancement in stability. The stabilizing mechanism is attributed to a combination of: (1) increased viscosity of the continuous phase, reducing the settling velocity of casein aggregates as per Stokes' law, and (2) potential electrostatic repulsion between charged protein particles, impeded by the anionic polysaccharide gum, preventing their aggregation and flocculation.

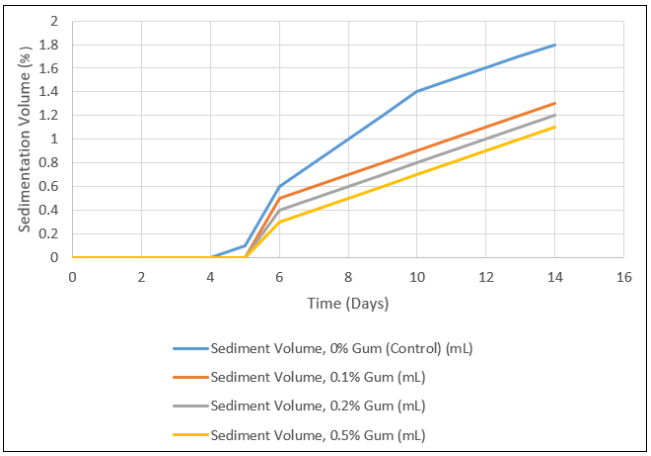

Fig 4: Sedimentation volume (mL) in milk-orange juice beverages containing different concentrations of L. leucocephala gum during 14 days of storage at 4°C

The pH of all beverages decreased slightly but significantly ($p < 0.05$) over the 30-day storage period, from an initial range of 4.72-4.86 to a final range of 4.40-4.47 (Table 4). This decline is likely due to residual microbial activity or

ongoing chemical acidification. Crucially, two-way ANOVA revealed that the rate of pH decline was not significantly influenced ($p > 0.05$) by the addition of the gum, indicating that the gum itself did not promote microbial spoilage or acidification.

Table 4: Change in pH of milk-orange juice during storage at 4°C

Treatment	Day 1	Day 10	Day 20	Day 30
0% Gum	4.86 ± 0.02^{aA}	4.70 ± 0.01^{bA}	4.58 ± 0.02^{cA}	4.47 ± 0.01^{dA}
0.1% Gum	4.80 ± 0.01^{aB}	4.67 ± 0.02^{bA}	4.55 ± 0.01^{cA}	4.45 ± 0.02^{dA}
0.2% Gum	4.75 ± 0.02^{ac}	4.65 ± 0.01^{bB}	4.53 ± 0.02^{cA}	4.42 ± 0.01^{dB}
0.5% Gum	4.72 ± 0.01^{ac}	4.63 ± 0.02^{bB}	4.51 ± 0.01^{cB}	4.40 ± 0.02^{dc}
Means with different lowercase superscripts (a-d) in the same row are significantly different ($p < 0.05$). Means with different uppercase superscripts (A-C) in the same column are significantly different ($p < 0.05$).				

3.5 Sensory Evaluation

The ultimate test of a new ingredient is its consumer acceptability. The mean sensory scores are presented in Table 5. Statistical analysis (one-way ANOVA) showed that for the attributes of color and aroma, there were no significant differences ($p > 0.05$) between the control and any of the gum-containing samples. This indicates that the off-white color and odorless nature of the gum did not alter the visual or olfactory profile of the beverage.

Table 5: Mean sensory scores (9-point hedonic scale) of milk-orange juice beverages

Attribute	0% Gum (Control)	0.1% Gum	0.2% Gum	0.5% Gum
Color	7.6 ± 0.8^{A}	7.9 ± 0.7^{A}	7.1 ± 1.0^{A}	6.7 ± 1.2^{A}
Aroma	6.9 ± 1.1^{A}	7.3 ± 0.9^{A}	6.8 ± 1.0^{A}	6.4 ± 1.3^{A}
Taste	8.7 ± 0.5^{aA}	8.9 ± 0.4^{aA}	7.4 ± 0.9^{bB}	6.5 ± 1.1^{cc}
Texture	8.8 ± 0.4^{aA}	8.3 ± 0.6^{aB}	7.7 ± 0.8^{bB}	7.5 ± 0.7^{bB}
Overall	$\mathbf{8.2 \pm 0.5^{aA}}$	$\mathbf{8.3 \pm 0.4^{aA}}$	$\mathbf{7.4 \pm 0.7^{bB}}$	$\mathbf{6.7 \pm 0.9^{cc}}$
Means in the same row with different lowercase superscripts (a-c) are significantly different ($p < 0.05$). Means in the same row with different uppercase superscripts (A-C) are significantly different ($p < 0.05$).				

For taste and texture, the beverages containing 0.1% and 0.2% gum were not statistically different from the control ($p > 0.05$), with scores for "taste" at 0.1% gum even slightly higher (8.9) than the control (8.7). This suggests that at these concentrations, the gum does not impart any off-flavors and contributes positively to the mouthfeel, likely by providing a fuller body. However, at the highest concentration (0.5%), there was a significant drop ($p < 0.05$) in scores for taste, texture, and overall acceptability. This is likely due to the high viscosity becoming perceptible as a slightly slimy or thick mouthfeel, and potentially the introduction of very subtle off-notes at high concentrations. Therefore, the optimal usage level for *L. leucocephala* gum in this application is determined to be between 0.1% and 0.2%.

4. Conclusion

This study successfully demonstrates that *Leucaena leucocephala* gum exudate is a highly effective natural stabilizer for acidified milk-orange juice beverages. The comprehensive characterization revealed it to be a high-purity, acidic polysaccharide with favorable functional and rheological properties, including pronounced shear-thinning behavior. Its incorporation at a 0.2% concentration significantly enhanced the physical stability of the beverage by reducing protein sedimentation over a 14-day shelf life. Most importantly, sensory evaluation confirmed that stabilization was achieved at organoleptically neutral concentrations, with no negative impact on the color, aroma, taste, or overall acceptability of the product compared to an unstabilized control.

L. leucocephala gum presents a sustainable, natural, and clean-label solution for the food industry, perfectly aligning with current consumer trends. It offers a viable value-added application for a widely available and often underutilized tree species. Future work should focus on detailed structural elucidation (monosaccharide composition, molecular weight distribution), toxicological studies to confirm its GRAS status, and exploring its efficacy in other complex food systems such as ice cream, sauces, and gluten-free products.

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