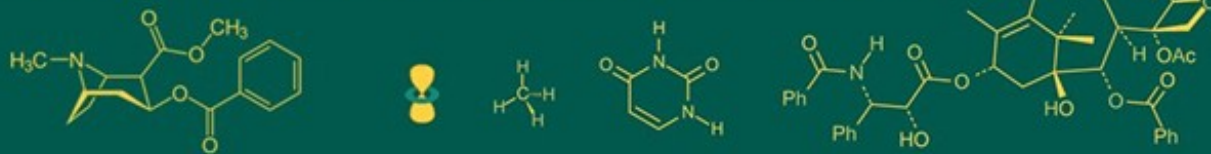


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Nutritional and functional quality, frozen-storage stability and economic feasibility of a mulberry–beetroot-enriched sugarcane juice ice popsicle

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ABSTRACT

The nutritional, functional, microbiological and economic performance of an optimized sugarcane juice popsicle containing mulberry, beetroot and ginger juices was evaluated against a sugarcane–ginger control. The optimized formulation (T_3) comprised 90% sugarcane juice, 6% mulberry juice, 2% beetroot juice and 2% ginger juice, whereas the control (T_0) contained 98% sugarcane juice and 2% ginger juice. Proximate composition, selected minerals, total phenolics, flavonoids, anthocyanins, betalain, ascorbic acid and antioxidant activity were determined. Microbiological quality was monitored at 0, 15, 30, 60 and 90 days during storage at -18°C , and production economics were calculated for a 10-L batch. T_3 contained 0.28% protein, 0.07% crude fibre and 0.33% ash, with an estimated energy value of 61.74 kcal/100 g. Phosphorus, zinc, magnesium, calcium, iron and potassium were marginally higher than in the control. Total phenolic content increased from 53.67 to 58.10 mg GAE/100 g, flavonoids from 13.78 to 15.12 mg QE/100 g, ascorbic acid from 2.84 to 3.45 mg/100 g and antioxidant activity from 41.25 to 52.84%. Anthocyanins (10.86 mg/100 g) and betalain (2.05 mg/100 g) were detected only in T_3 . Total plate counts remained undetected through 30 days and reached 3.0×10^2 CFU/g in both products at 90 days, while coliforms were not detected throughout storage. The production cost was ₹7.19 per enriched popsicle compared with ₹3.76 for the control. The optimized product provided natural pigments and higher antioxidant activity and demonstrated microbiological stability under the evaluated frozen-storage conditions for 90 days, while its higher production cost was primarily associated with the inclusion of mulberry juice.

Keywords: Sugarcane juice, Mulberry, Beetroot, Anthocyanins, Betalain, Antioxidant activity, Frozen storage, Microbiological quality, Production cost

Introduction

The development of frozen products from fruit and vegetable juices provides an opportunity to combine convenience, sensory appeal and plant-derived functional constituents. Sugarcane juice is naturally sweet and suitable as the principal phase of an ice popsicle, but its nutritional profile is dominated by water and carbohydrates. Incorporation of strongly coloured juices can diversify this matrix by supplying natural pigments, phenolic compounds and micronutrients. The technological value of such enrichment, however, must be assessed together with microbiological stability and production cost. A formulation may show improved laboratory composition yet remain commercially unattractive or unstable during storage.

Mulberry juice contributes anthocyanins, especially cyanidin derivatives, that provide red–purple colour and participate in antioxidant reactions (Du *et al.*, 2008; Aramwit *et al.*, 2010) [2, 1]. Beetroot supplies betalain pigments, which are water-soluble nitrogen-containing compounds with colouring and radical-scavenging properties (Sadowska-Bartoszyk *et al.*, 2021) [3]. Ginger contains phenolic constituents and offers a concentrated aroma contribution even at a low formulation level. Combining these ingredients with sugarcane juice may therefore produce a more functionally diverse frozen product without relying on synthetic colourants.

The functional significance of enrichment cannot be inferred merely from the known composition of the ingredients. Dilution, extraction, blending and freezing determine the concentration ultimately present in the edible product. Direct comparison between a control and optimized formulation is needed to quantify changes in proximate composition, minerals, phenolics, flavonoids, pigments, ascorbic acid and antioxidant activity. Pigment-specific measurements are particularly valuable because the appearance of anthocyanins and betalains in the final product provides traceable evidence of the functional contribution of mulberry and beetroot.

Frozen storage suppresses microbial multiplication but does not sterilize a product. Contamination introduced through fresh juice extraction, handling, mould filling or packaging can survive at low temperature, and psychrotolerant microorganisms or yeasts and moulds may become detectable during extended storage.

Monitoring total plate count, coliforms, and yeast and mould counts is therefore necessary when assessing storage quality. In parallel, techno-economic analysis identifies whether nutritional and functional improvements can be achieved at a plausible per-unit cost.

The objective of this study was to compare the control and sensory-optimized sugarcane juice popsicles for proximate composition, theoretical energy, selected minerals, phytochemical constituents and antioxidant activity; evaluate their microbiological quality over 90 days at -18°C ; and determine the production cost of both formulations. Raw-material characterization and formulation optimization were outside the scope of this manuscript.

Materials and Methods

Product formulations and preparation

Two previously standardized formulations were used. The control (T_0) contained 98% sugarcane juice and 2% ginger juice. The optimized treatment (T_3) contained 90% sugarcane juice, 6% mulberry juice, 2% beetroot juice and 2% ginger juice. Xanthan gum and citric acid were added at the common standardized levels used in the experiment. The ingredients were blended, filled into sanitized cylindrical moulds and frozen at -18°C . The products were prepared using standardized laboratory procedures, with optimized levels of stabilizer and acidulant. After filling and freezing under controlled conditions, the popsicles were packaged and stored at -18°C for subsequent analyses.

2. Proximate composition and energy value

Moisture, protein, carbohydrate, crude fibre, fat and ash contents were determined in triplicate using standard analytical procedures. Moisture, protein, crude fibre, fat and ash were determined by standard proximate analysis methods, while carbohydrate content was calculated by difference. Theoretical energy was calculated using Atwater factors: 4 kcal/g for carbohydrate, 4 kcal/g for protein and 9 kcal/g for fat. The results were expressed on a per 100 g basis.

Mineral analysis

Phosphorus, zinc, magnesium, calcium, iron and potassium were determined after acid digestion of the samples. The digested samples were analyzed using an atomic absorption spectrophotometer (AAS) at the respective analytical wavelengths for each mineral. Calibration was performed using appropriate certified standard solutions, and the mineral concentrations were calculated from the corresponding calibration curves. Results were expressed as mg/100 g of sample. All analyses were carried out in triplicate.

Phytochemical and antioxidant analyses

Total phenolic content was expressed as mg gallic acid equivalents (GAE)/100 g, and total flavonoid content as mg quercetin equivalents (QE)/100 g. Total anthocyanin, betalain and ascorbic acid contents were expressed as mg/100 g. Antioxidant activity was expressed as percentage radical-scavenging activity according to the respective assay. All determinations were carried out in triplicate, and the results were expressed as mean values with appropriate measures of variation.

2.5 Frozen-storage microbiological analysis

Packaged control and optimized popsicles were stored at -18°C and analyzed at 0, 15, 30, 60 and 90 days of storage. Representative samples were aseptically homogenized in sterile diluent and serially diluted for microbiological enumeration. Total plate count was determined using Plate Count Agar, yeast and mould count using Potato Dextrose Agar, and coliform count using the appropriate microbiological medium and standard procedure. The plates were incubated under the prescribed conditions for each microbial group. Microbial counts were expressed as CFU/g. Results below the analytical detection limit were reported as not detected (ND).

6. Techno-economic analysis

Production cost was calculated for 10 L of mixture. Raw-material expenditure was based on the prevailing purchase price and quantity used. Processing cost was estimated as 20% of raw-material cost. Packaging was calculated at ₹1.00 per popsicle, and 180 popsicles were obtained from each 10-L batch. Total cost and cost per popsicle were determined. This estimate represents small-scale production and excludes capital depreciation, labour allocation beyond the processing percentage, distribution, marketing, profit margin and taxes.

Statistical analysis

All analytical determinations were carried out in triplicate, and the results were expressed as mean $\pm$ standard deviation (SD). The data for the control (T_0) and optimized formulation (T_3) were statistically analyzed using an independent-samples *t*-test at a significance level of $p < 0.05$. Microbiological counts were expressed as CFU/g, and non-detectable (ND) values were reported as such and were not considered as zero values for statistical interpretation. Statistical analysis was performed using IBM SPSS Statistics software.

Results and Discussion

Proximate composition and energy value

Enrichment produced small but directionally consistent changes in proximate composition (Table 1). Moisture increased from 83.94 to 84.29%, while carbohydrate decreased from 15.37 to 14.93%. The substitution of part of the carbohydrate-rich sugarcane juice with mulberry, beetroot and ginger juices explains both responses. Protein increased from 0.24 to 0.28%, crude fibre from 0.05 to 0.07%, fat from 0.08 to 0.10% and ash from 0.32 to 0.33%. Although numerically modest, these changes demonstrate that enrichment diversified the matrix rather than substantially increasing macronutrient density.

Optimized (T_3)

Table 1. Proximate composition and theoretical energy value of sugarcane juice popsicles

Parameter	Control (T_0)	
Moisture (%)	83.94 $\pm$ 0.34	
	84.29 $\pm$ 0.39	
Protein (%)	0.24 $\pm$ 0.02	0.28 $\pm$ 0.03
Carbohydrate (%)	15.37 $\pm$ 0.27	14.93 $\pm$ 0.24
Crude fibre (%)	0.05 $\pm$ 0.01	0.07 $\pm$ 0.02
Fat (%)	0.08 $\pm$ 0.02	0.10 $\pm$ 0.01
Ash (%)	0.32 $\pm$ 0.03	0.33 $\pm$ 0.02
Energy (kcal/100 g)	63.16	61.74

Values are mean $\pm$ SD ($n = 3$), unless otherwise stated.

The calculated energy value decreased by 1.42 kcal/100 g, from 63.16 in the control to

61.74 in T_3 . This difference was mainly associated with lower carbohydrate-derived energy and was only partly offset by the small increases in protein and fat. The optimized popsicle should therefore be described as functionally enriched rather than as a major source of protein or fibre. Likewise, the 2.2% reduction in energy does not justify a low-energy claim without comparison against the applicable regulatory criteria.

Mineral composition

All measured minerals were slightly higher in T_3 than in T_0 (Table 2). Phosphorus increased from 20.33 to 21.72 mg/100 g, magnesium from 12.73 to 13.32 mg/100 g and calcium from 18.01 to 19.33 mg/100 g. Zinc and iron showed smaller absolute changes, while potassium—the predominant measured mineral—increased

from 164.15 to 170.00 mg/100 g. These increments reflect the combined contribution of the incorporated juices, but they remain modest because 90% of the optimized formulation was still sugarcane juice.

Table 2. Mineral composition of control and optimized sugarcane juice popsicles

Mineral (mg/100 g)	Control (T ₀)	Optimized (T ₃)
Phosphorus	20.33 ± 0.71	21.72 ± 0.76
Zinc	0.12 ± 0.004	0.13 ± 0.005
Magnesium	12.73 ± 0.45	13.32 ± 0.47
Calcium	18.01 ± 0.63	19.33 ± 0.68
Iron	1.08 ± 0.04	1.12 ± 0.04
Potassium	164.15 ± 2.50	170.00 ± 2.80

Values are mean ± SD (n = 3), unless otherwise stated.

The mineral findings should not be translated into claims regarding bone health, blood formation or electrolyte replacement without calculating serving contribution relative to regulatory reference values. Their scientific value lies in demonstrating compositional retention and a broad, measurable enhancement rather than therapeutic functionality.

Phytochemical composition and antioxidant activity

The clearest effect of enrichment was observed in the phytochemical profile (Table 3). Total phenolics increased by approximately 8.3%, from 53.67 to 58.10 mg GAE/100 g, and total flavonoids increased by approximately 9.7%, from 13.78 to 15.12 mg QE/100 g. Ascorbic acid rose by approximately 21.5%, reaching 3.45 mg/100 g. The higher values demonstrate that the added juices contributed compounds beyond sugars and organic acids.

Table 3. Phytochemical composition and antioxidant activity of sugarcane juice popsicles

Parameter	Control (T ₀)	Optimized (T ₃)
Total phenolics (mg GAE/100 g)	53.67 ± 1.85	58.10 ± 2.10
Total flavonoids (mg QE/100 g)	13.78 ± 0.62	15.12 ± 0.71
Anthocyanins (mg/100 g)	ND	10.86 ± 0.48
Betalain (mg/100 g)	ND	2.05 ± 0.12
Ascorbic acid (mg/100 g)	2.84 ± 0.15	3.45 ± 0.18
Antioxidant activity (%)	41.25 ± 1.95	52.84 ± 2.30

Values are mean ± SD (n = 3). ND, not detected. GAE, gallic acid equivalents; QE, quercetin equivalents.

Anthocyanins and betalain were not detected in the control but were quantified at 10.86 and 2.05 mg/100 g, respectively, in T₃. Their appearance provides direct compositional evidence of mulberry and beetroot incorporation. Mulberry anthocyanins are reported to contain cyanidin glycosides, whereas beetroot betalains are chemically distinct water-soluble pigments (Du *et al.*, 2008; Sadowska-Bartos *et al.*, 2021) [2, 3]. Because the two pigment classes can respond differently to pH, oxygen, light and temperature, their retention during longer storage should be investigated separately.

Antioxidant activity increased from 41.25 to 52.84%, corresponding to an improvement of approximately 28.1%. The proportional increase was greater than that of total phenolics or flavonoids alone, suggesting that anthocyanins, betalains, ascorbic acid and other con-

stituents jointly contributed to radical-scavenging performance. This should be interpreted as an analytical antioxidant response rather than evidence of a clinical health effect. Correlation analysis and the use of an additional antioxidant assay would strengthen the mechanistic interpretation.

Microbiological quality during frozen storage

No total plate count, coliforms, or yeast and mould were detected in either product at 0, 15

or 30 days (Table 4). At 60 days, total plate counts became detectable at 1.8×10^2 CFU/g in T₀ and

2.0×10^2 CFU/g in T₃, whereas coliforms and yeast and mould remained undetected. At 90 days, the total plate count reached 3.0×10^2 CFU/g in both products. Yeast and mould appeared only at low levels—4 CFU/g in the control and 5 CFU/g in T₃—and coliforms remained undetected throughout.

Table 4. Microbiological quality of sugarcane juice popsicles during storage at -18 °C

Storage (days)	TPC T ₀ (CFU/g)	TPC T ₃ (CFU/g)	Coliform T ₀ /T ₃	Yeast-mould T ₀	Yeast-mould T ₃
0	ND	ND	ND / ND	ND	ND
15	ND	ND	ND / ND	ND	ND
30	ND	ND	ND / ND	ND	ND
60	1.8×10^2	2.0×10^2	ND / ND	ND	ND
90	3.0×10^2	3.0×10^2	ND / ND	4.0×10^0	5.0×10^0

TPC, total plate count; ND, not detected at the analytical detection limit.

The delayed appearance of microbial counts reflects the inhibitory influence of continuous frozen storage, hygienic preparation and the acidic product environment. Freezing inhibits microbial growth but may permit the survival of some microorganisms. Therefore, the increase in counts during later storage may be associated with the recovery of surviving microorganisms, sampling variability or minor temperature fluctuations. The absence of coliforms throughout the storage period indicates satisfactory hygienic quality under the tested conditions; however, it does not confirm the complete absence of foodborne pathogens.

The microbial counts remained low throughout the 90-day storage period. The total plate counts at 90 days (3.0×10^2 CFU/g in both T₀ and T₃) were below the aerobic plate count limit specified by FSSAI for the category covering ice cream, frozen dessert, milk lolly and ice candy. Coliforms were not detected throughout storage and were therefore below the corresponding FSSAI limit. Yeast and mould were detected only at low levels at 90 days (4 CFU/g in T₀ and 5 CFU/g in T₃); however, no specific criterion for yeast and mould is assigned for this product category in the cited FSSAI Appendix B.

Thus, the evaluated microbiological parameters were within the applicable FSSAI criteria where such criteria were specified, and both formulations demonstrated microbiological stability under the evaluated frozen-storage conditions for 90 days. This interpretation is limited to

the microorganisms and analytical parameters evaluated in the present study and should not be taken as evidence of absence of all foodborne pathogens.

Techno-economic feasibility

The optimized formulation was more expensive than the control (Table 5). Raw-material expenditure increased from ₹414.36 to ₹928.36 per 10 L, primarily because 600 mL of mulberry juice cost ₹540.00. Processing cost, calculated at 20% of raw-material expenditure, consequently rose from ₹82.87 to ₹185.67. Packaging remained ₹180.00 for both batches because each produced 180 popsicles.

Table 5. Comparative techno-economic feasibility of control and optimized popsicles

Economic parameter	Control (T ₀)	Optimized (T ₃)
Raw-material cost/10 L (₹)	414.36	928.36
Processing cost/10 L (₹)	82.87	185.67
Packaging cost/10 L (₹)	180.00	180.00
Total production cost/10 L (₹)	677.23	1,294.03
Popsicles produced (number)	180	180
Production cost/popsicle (₹)	3.76	7.19

Processing cost was estimated as 20% of raw-material cost; packaging was ₹1.00 per popsicle. Values represent the study's small-scale costing assumptions.

Total production cost increased from ₹677.23 to ₹1,294.03 per 10 L, and unit cost increased by ₹3.43, or approximately 91%, from ₹3.76 to ₹7.19. The yield remained identical, showing that enrichment affected ingredient expenditure rather than number of units produced. Mulberry juice was the dominant cost driver and accounted for about 58% of the optimized raw-material cost. The economic viability of T₃ therefore depends on whether consumers are willing to pay for natural pigmentation and greater functional differentiation.

The absolute unit cost remained compatible with a small packaged frozen product, but the analysis is preliminary. A commercialization decision would require labour, utilities, depreciation, cold-chain distribution, wastage, retailer margin, taxes and sensitivity to seasonal mulberry prices. Local sourcing, use of frozen mulberry concentrate or optimization of pigment extraction could be explored to reduce cost while preserving product quality.

Integrated assessment and study limitations

T₃ offered a coherent value proposition: it introduced two identifiable natural pigment classes, increased measured antioxidant activity, produced modest mineral and compositional gains, and maintained low microbial counts during 90 days of frozen storage. These improvements were achieved without a substantial change in energy value. The principal trade-off was economic, with production cost nearly doubling because of mulberry juice.

The study has limitations that should be acknowledged. Nutritional and functional comparisons involved only T₀ and T₃, the microbiological study did not include pathogen challenge testing, and storage evaluation did not measure pigment, antioxidant, physicochemical or sensory retention at each interval. Colour and texture stability therefore cannot be inferred from microbiological stability. The cost analysis was based on small-scale assumptions and did not include a com-

plete business model. Future work should combine microbiological monitoring with anthocyanin, betalain, ascorbic acid, antioxidant activity, pH, colour and sensory measurements throughout frozen storage.

Conclusion

The optimized sugarcane juice popsicle containing 6% mulberry, 2% beetroot and 2% ginger juices showed a more diverse functional profile than the sugarcane–ginger control. It contained measurable anthocyanins and betalain, higher phenolic, flavonoid and ascorbic acid contents, and approximately 28% greater antioxidant activity. Mineral increases were consistent but modest, while energy decreased slightly to 61.74 kcal/100 g. Both formulations maintained low microbial counts during 90 days at –18 °C, and coliforms were not detected. Enrichment increased the production cost from ₹3.76 to ₹7.19 per popsicle, mainly because of mulberry juice. T₃ combined functional differentiation with microbiological stability under the evaluated frozen-storage conditions

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