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PHYSICO-CHEMICAL STABILITY AND MICROBIOLOGICAL SAFETY OF NOVEL WOOD APPLE (*LIMONIA ACIDISSIMA L.*) NECTAR BEVERAGES

Food Technology

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ABSTRACT

The present study investigates the physico-chemical characteristics and microbiological safety of novel wood apple (*Limonia acidissima L.*) nectar beverages developed using different formulations. Four variants were prepared, namely N1 (wood apple nectar), N2 (wood apple nectar with pineapple), N3 (wood apple nectar with pomegranate), and N4 (wood apple nectar with spices), and evaluated under baseline and accelerated storage conditions equivalent to 90 days. Physico-chemical parameters including total soluble solids (°Brix), total acidity, and pH were analyzed using standard AOAC methods, while microbiological quality was assessed in accordance with FSSR (2011) guidelines. The results revealed that total soluble solids ranged from 18.5 to 20.25°Brix, with the highest values observed in the pomegranate-based formulation (N3). Total acidity varied between 0.83% and 0.93%, with relatively higher acidity in N2 due to pineapple incorporation. The pH values (3.50–3.64) indicated a stable acidic environment conducive to product preservation. Minimal variations in physico-chemical parameters under accelerated storage conditions confirmed good product stability. Microbiological analysis showed negligible aerobic microbial counts (<1 cfu/ml), absence of yeast and mould, and complete absence of major foodborne pathogens, including Escherichia coli, Salmonella, Listeria monocytogenes, and Staphylococcus aureus, throughout the storage period. Overall, the developed wood apple nectar beverages demonstrated excellent physicochemical stability and microbiological safety, meeting regulatory standards and indicating strong potential for commercial production as functional, value-added fruit beverages.

KEYWORDS

Wood apple (*Limonia acidissima),* Nectar formulation, Physico-chemical properties, Microbiological quality, Shelf-life stability, Accelerated storage, Food safety, Value-added products, Underutilized fruits.

1. INTRODUCTION

Wild fruits are becoming rarer to find over time due to multiple reasons like deforestation, natural landscape conversions, changes in climate, altering of food habits towards more commercially viable varieties, gaps in transfer of traditional knowledge through generations, changes in taste preferences, industrialization of agriculture, resources and time required, and uncertainty of returns if a wild crop is selected for a crop cycle, lack of knowledge on nutritional and health uses which in a way is proportionating to lack of demand, the harmful effects of agricultural pesticides on wild pollinators and natural ecosystem that wild fruits depend on for reproduction. Due to all such reasons which are making wild fruits availability rare, many wild fruits and associated nutritional advantages and sensory experiences are slowly vanishing from the current generations.

The current work is targeted on development novel wild fruit nectar beverages with Wood apple (*Limonia acidissima*). Wood apple is an underutilized medicinal and nutraceutical fruit belonging to the Rutaceae family, traditionally employed in the treatment of diabetes, diarrhea, ulcers, cancer, and microbial infections. Its therapeutic properties are largely attributed to a diverse range of bioactive phytochemicals, including flavonoids, polyphenols, alkaloids, glycosides, tannins, and essential oils.

There is growing consumer demand for natural, traditional, and functional beverages as alternatives to carbonated soft drinks. Wood apple beverages can be positioned as ethnic, heritage, or wellness-oriented products without making medicinal claims, aligning well with current market trends. The beverage format also supports portion control, standardization, branding, and distribution through existing retail channels, which is difficult to achieve with fresh or minimally processed fruits.

1.1 Aim

To methodically analyse physico-chemical and microbial parameters of novel Wood Apple Nectar beverages.

1.2 Objectives

1. To analyze physico-chemical properties of the developed nectar beverages for an ambient storage period equivalent of 90 days, by accelerated shelf-life study.
2. To analyze microbial loads of the developed beverages for an ambient storage period of 90 days.

2. REVIEW OF LITERATURE

Wood apple (*Limonia acidissima L.*) is an underutilized, nutritionally rich fruit with significant potential for functional food development, value addition, and therapeutic applications. Its pulp, leaves, and seeds are rich in macronutrients, micronutrients, phytochemicals, and bioactive compounds such as polyphenols, flavonoids, and essential fatty acids, contributing to antioxidant, antibacterial, anti-inflammatory, and medicinal properties (Pandey et al., 2014; Dyuti et al., 2022; Kerkar et al., 2020; Vijayvargia & Vijayvergia, 2014; Rodrigues et al., 2018). Advanced extraction techniques such as ultrasound-assisted extraction have further enhanced the recovery of bioactive compounds with high antioxidant potential, indicating its relevance in nutraceutical applications (Hoa et al.). Studies on physical, chemical, and compositional characteristics highlight its suitability for processing, despite limitations in mechanization and post-harvest handling (Khan et al., 2019; Ramakrishna et al., 1979).

Processing techniques, particularly drying, play a crucial role in determining the nutritional and functional quality of wood apple products. Tray drying has been reported to retain higher levels of protein, carbohydrates, and antioxidant activity compared to microwave drying, while solar drying enhances polyphenol content (Das et al., 2022; Vijayakumar et al., 2013). Nutrient concentration generally increases during drying, although heat-sensitive compounds like vitamin C may degrade. Such findings support the importance of optimizing processing conditions for quality retention.

Extensive research has been conducted on value-added product development from wood apple, including jellies, RTS beverages, sauces, pickles, toffees, powders, spreads, cookies, and innovative products such as whey-based protein beverages and emergency nutrition jellies (Khan et al., 2019; Anitha et al., 2023; Akhil Verma et al., 2023; Jahagirdar et al., 2022; Hemalatha & Parameshwari, 2019; Puvanendran et al., 2024; Afifah et al., 2023). Optimization studies indicate that formulation variables such as pulp ratio, sugar concentration, and blending with other fruits (e.g., guava, karonda) or ingredients (ginger, cardamom, whey) significantly influence physicochemical properties, sensory attributes, and nutritional quality (Amardeepa & Vijayakumar, 2023; Verma & Deen, 2024; Ishan & Deen, 2025; Lohakare et al., 2025). Blended formulations often improve acceptability and nutritional enhancement, with specific ratios yielding superior organoleptic scores.

Storage studies consistently reveal similar trends across products, including increases in total soluble solids (TSS), acidity, sugars, and browning, along with decreases in ascorbic acid, pH, and sensory

quality over time, although microbial loads generally remain within acceptable limits for defined storage periods (Awadhesh Kumar & Deen; Moazzem et al., 2019; Kore et al., 2025). Shelf-life extension techniques, including the use of preservatives and pasteurization, have proven effective in maintaining product safety and quality. Additionally, sugar optimization in fruit-based beverages is important for balancing sensory acceptability and health considerations (Oliveira et al., 2018).

Beyond processing, wood apple is recognized for its ecological, economic, and dietary significance as a wild edible fruit with potential to improve food and nutritional security, particularly in underdeveloped regions (Bvenura & Sivakumar, 2017; Suwardi et al., 2026). Despite its high potential, its utilization remains limited due to lack of awareness, processing technologies, and commercialization strategies. Overall, the literature strongly indicates that wood apple is a promising candidate for functional food development, nutraceutical applications, and sustainable food systems, though further research is required in process optimization, product standardization, and large-scale commercialization.

3. MATERIALS & METHODS

Standardized novel wood apple nectar beverages are analyzed for physico-chemical and microbial parameters, baseline and for a shelf-life equivalent of 90 days.

Four types of wood apple nectar beverages are developed with 20 to 30% fruit content justifying the fruit percentage requirement for nectars as per FSSAI standards.

N-1 – Wood apple nectar
N-2 – Wood apple nectar with Pineapple
N-3 – Wood apple nectar with Pomegranate
N-4 – Wood apple nectar with Spices

The developed products contain water, wood apple pulp, pineapple juice, pomegranate juice, jaggery, red chilli powder, salt, food grade citric acid, food grade class-II preservative potassium sorbate. Table-1 provides ingredient mapping of nectar variants.

Table 1 : Ingredient mapping of Wood apple nectar beverages

S.no	Ingredient details	N-1	N-2	N-3	N-4
1	Wood apple pulp	Yes	Yes	Yes	Yes
2	Pineapple juice	No	Yes	No	No
3	Pomegranate juice	Yes	No	No	No
4	Chilli powder	No	No	No	Yes
5	Jaggery	Yes	Yes	Yes	Yes
6	Salt	Yes	Yes	Yes	Yes
7	Citric acid anhydrous	Yes	Yes	Yes	Yes
8	Potassium sorbate	Yes	Yes	Yes	Yes
9	Water	Yes	Yes	Yes	Yes

3.1 Physico-chemical analyses

The products are analyzed for Brix (%), Total acidity (as citric acid) (%) and pH as per approved methods.

For shelf-life study, the mentioned physico-chemical parameters are analysed under accelerated incubated conditions at 40±3ºC 75±5% RH, for 30 days giving an equivalence to 90 days at ambient conditions.

3.2 Microbial analyses

All 4 variants of wood apple nectar beverages are analyzed for microbial parameters Aerobic microbial count, E.Coli O157 : H7, Enterobacteriaceae, Listeria monocytogenes, Vibrio cholera, Salmonella, Staphylococcus aureus, Sulphite-reducing clostridia and Yeast & mould, with approved standard methods.

4. RESULTS & DISCUSSION

4.1 The results of physico-chemical analysis are tabulated below in table-2.

Table 2 : Physico-chemical parameters analysis of Wood apple nectar beverages

S. no.	Test parameter	Test method	LOQ	N1		N2		N3		N4	
				Baseline (B)	Accelerated (A)	B	A	B	A	B	A
1	Brix (%)	AOAC 21st Ed. 932.14	0.1	18.5	18.75	19.75	19.5	20.25	20.25	18.75	19.25
2	Total acidity (%)	AOAC 21st Ed. 942.15	0.1	0.85	0.83	0.93	0.93	0.89	0.88	0.87	0.87
3	pH	AOAC 21st Ed. 981.12	1	3.57	3.58	3.64	3.63	3.62	3.63	3.52	3.5

The physicochemical properties of developed wood apple nectar variants (N1–N4) were evaluated under baseline (B) and accelerated (A) storage conditions, and the results are presented in terms of total soluble solids (°Brix), total acidity, and pH.

4.1.1 Total Soluble Solids (°Brix)

The total soluble solids of the beverages ranged from 18.5 to 20.25°Brix across all formulations. At baseline conditions, N3 (wood apple nectar with pomegranate) exhibited the highest °Brix (20.25°), followed by N2 (19.75°), N4 (18.75°), and N1 (18.5°). Under accelerated conditions, slight variations were observed, with N3 maintaining the highest value (20.25°), while N2 showed a marginal decrease (19.5°). N1 and N4 exhibited a minor increase to 18.75° and 19.25°, respectively. The relatively higher °Brix in N2 and N3 may be attributed to the natural sugars contributed by pineapple and pomegranate, respectively. The minimal changes under accelerated storage indicate good stability of soluble solids, suggesting limited degradation or concentration effects during storage. These findings are consistent with typical fruit beverage behaviour.

4.1.2 Total Acidity (%)

Total acidity values ranged from 0.83% to 0.93% across all samples. At baseline, N2 showed the highest acidity (0.93%), followed by N3 (0.89%), N4 (0.87%), and N1 (0.85%). Under accelerated conditions, acidity values remained nearly constant, with only slight reductions observed in N1 (0.83%) and N3 (0.88%), while N2 and N4 remained unchanged. The higher acidity in N2 can be linked to the inherent organic acid content of pineapple, while pomegranate also contributed to relatively elevated acidity in N3. The negligible variation during accelerated storage suggests good acid stability and absence of significant biochemical or microbial degradation affecting acid composition.

4.1.3 pH

The pH of the beverages ranged between 3.50 and 3.64, indicating that all formulations fall within the acidic range favorable for microbial stability. At baseline, N2 recorded the highest pH (3.64), followed by N3 (3.62), N1 (3.57), and N4 (3.52). Under accelerated conditions, pH values showed minimal fluctuation, with a slight increase in N1 and N3 and a marginal decrease in N4.

The inverse relationship between acidity and pH was evident across formulations. The stability of pH under accelerated storage further confirms that no major acid degradation or microbial spoilage occurred. The consistently low pH values (<4.0) are advantageous for extending shelf life by inhibiting pathogenic microbial growth.

Overall Interpretation

Across all parameters, the developed wood apple nectar variants demonstrated good physicochemical stability under accelerated storage conditions. Among the formulations, N3 (wood apple with pomegranate) showed superior performance in terms of higher soluble solids and balanced acidity, while N2 exhibited the highest acidity levels. The stability of °Brix, acidity, and pH suggests that the beverages are well-formulated and suitable for storage without significant quality deterioration.

4.2 Microbiological Quality and Safety Evaluation

The microbiological quality of wood apple-based beverages (N1–N4) was assessed at initial (0th day) and after 90 days of storage (accelerated equivalent), and the results are presented in Table 3.

The analysis included total aerobic microbial count, yeast and mould, and major foodborne pathogens, in accordance with FSSR (2011) standards.

4.2.1 Total Aerobic Microbial Count

The total aerobic microbial count in all formulations was below the limit of quantification (<1 cfu/ml) at both initial and 90th day of storage. These values are significantly lower than the permissible limit (1×10 cfu/ml) specified under FSSR guidelines.

S.no.	Test parameter	Unit	LOQ	Spec. per FSSR	Test method	N1		N2		N3		N4	
						0th day result	90th day result	0th day	90th day	0th day result	90th day result	0th day result	90th day result
1	Aerobic microbial count	cfu/ml	1	M=1*10 4	IS 5402 (P.1):2021	<1	<1	<1	<1	<1	<1	<1	<1
2	E.Coli O157 : H7	Per 25ml	-	Absent	IS 14397:1996	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
3	Enterobacteriaceae	Per ml	-	Not detectable	ISO 21528 (P-1):2017	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
4	Listeria monocytogenes	Per 25ml	-	Absent	IS 14988 (P.1):2020	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
5	Vibrio cholera	Per 25ml	-	Absent	IS 5887 (P.5):1976	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
6	Salmonella	Per 25ml	-	Absent	IS 5887(P.3) sec.1 : 2020	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
7	Staphylococcus aureus	Per 25ml	-	Absent	IS 5887 (P.2):1976	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
8	Sulphite-reducing clostridia	cfu/ml	1	M=NA	ISO: 15213:2023	<1	<1	<1	<1	<1	<1	<1	<1
9	Yeast & mould	cfu/ml	1	M=1*10 3	IS 5403:1999	<1	<1	<1	<1	<1	<1	<1	<1

The negligible microbial load indicates effective thermal processing and hygienic handling, minimal post-processing contamination, strong inhibitory conditions within the beverage matrix. The consistently low counts during storage further confirm the microbiological stability of all formulations under accelerated conditions.

4.2.2 Yeast and Mould Count

Yeast and mould counts were also found to be <1 cfu/ml across all samples throughout storage, well below the maximum allowable limit (1×10^3 cfu/ml).

This observation is particularly significant because yeast and mould are the primary spoilage organisms in fruit-based beverages. Their absence suggests effective control of osmotic pressure and acidity, absence of favorable conditions for fungal growth, adequate packaging integrity preventing contamination. The results strongly indicate extended shelf-life stability without fungal spoilage.

4.2.3 Foodborne Pathogens

All tested pathogenic microorganisms were absent in 25 ml samples for both initial and stored conditions across all formulations, including Escherichia coli O157:H7, Enterobacteriaceae, Listeria monocytogenes, Vibrio cholerae, Salmonella spp., Staphylococcus aureus. The complete absence of these pathogens confirms compliance with food safety standards, effectiveness of processing interventions (heat treatment, sanitation), suitability of the beverages for safe human consumption.

4.2.4 Sulphite-Reducing Clostridia

Sulphite-reducing clostridia were below detectable limits (<1 cfu/ml) in all samples throughout storage. This is critical because these organisms are associated with anaerobic spoilage and toxin production.

Their absence suggests effective elimination of spore-forming anaerobes, adequate processing and storage conditions preventing germination and growth.

4.2.5 Effect of Formulation on Microbial Stability

No variation in microbiological quality was observed among the different formulations (N1–N4), indicating that incorporation of pineapple, pomegranate, and spices did not introduce microbial risk.

The acidic environment (pH < 4.0) formulation with addition of class-II preservative and appropriate processing and filling methods across all samples played a dominant role in inhibiting microbial growth.

4.2.6 Stability During Storage

The absence of microbial growth even after 90 days equivalent storage demonstrates high microbiological shelf stability, no post-processing contamination or microbial proliferation. Strong preservation due to acidic pH and addition of class-II preservative at allowed limits. These findings are consistent with the observed physicochemical accelerated studies, further validating product stability. storage validation confirms commercial shelf-life feasibility.

4.2.7 Compliance with Regulatory Standards and Industrial Relevance

All formulations complied fully with FSSR (2011) microbiological standards, indicating their suitability for commercialization. The results confirm that the developed beverages meet regulatory safety requirements, exhibit long shelf life without microbial spoilage, can be positioned as safe, ready-to-drink exotic wild-fruit beverages.

5. CONCLUSION

The present study successfully developed and evaluated novel wood apple nectar beverages with different formulations and demonstrated their physicochemical stability and microbiological safety under accelerated storage conditions equivalent to 90 days. All formulations (N1–N4) exhibited stable total soluble solids, acidity, and pH, with only minimal variations during storage, indicating robustness of the developed products.

Among the variants, the pomegranate-based formulation (N3) showed comparatively higher total soluble solids and balanced acidity, while the pineapple-based formulation (N2) exhibited higher acidity levels. The consistently low pH (<4.0) across all samples contributed significantly to microbial inhibition and product preservation. Microbiological analysis confirmed the absence of spoilage organisms and pathogenic bacteria throughout storage, with all parameters well within FSSR (2011) permissible limits, highlighting the effectiveness of processing, formulation, and preservation strategies.

The findings establish that incorporation of complementary ingredients such as pineapple, pomegranate, and spices enhances product diversity without compromising safety or stability. The developed beverages can be positioned as safe, functional, and commercially viable ready-to-drink products derived from an underutilized wild fruit. This study supports the potential for large-scale production and promotes the utilization of wood apple in value-added food applications, contributing to nutritional security and sustainable food systems.

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