



doi:10.5281/zenodo.20356926

Microbial Safety and Quality of Commercially Available Packaged Fruit Juices in Nigeria: Microbiological Assessment and Implications for High-Pressure Processing

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ABSTRACT

The microbial safety of three commercially available packaged fruit juice brands sold in Nigeria, Exotic, 5 Alive, and Capri-Son was evaluated through heterotrophic plate counts, selective media enumeration, Gram staining, and standard biochemical characterization. Total viable bacterial counts ranged from 2.37×10^3 to 3.60×10^3 CFU/mL across nine subsamples (three replicates per brand), representing low-to-moderate microbial loads. Growth was recovered on MacConkey, Mannitol Salt, Salmonella-Shigella, and de Man-Rogosa-Sharpe (MRS) agars, but not on Cetrinide or Thiosulfate-Citrate-Bile-Sucrose (TCBS) agars, indicating the absence of *Pseudomonas* and *Vibrio* spp. A total of 81 isolates were recovered and presumptively identified; *Lactobacillus* spp. was most prevalent (24.69%), followed by *Bacillus cereus* and *Staphylococcus aureus* (both 14.81%), *Bacillus subtilis* (12.35%), *Staphylococcus epidermidis* (9.88%), *Enterobacter* spp. and *Salmonella* spp. (both 8.64%), and *Shigella* spp. (6.17%). The detection of recognized enteric pathogens and toxin-producing organisms in products marketed as pasteurized indicates limitations in current thermal processing efficacy or post-processing contamination. These findings support the case for adopting non-thermal technologies notably high-pressure processing (HPP) at 400–600 MPa to achieve the regulatory ≥ 5 -log pathogen reduction required for acidic juice matrices while preserving sensory and nutritional attributes.

Keywords: fruit juice microbiology; high-pressure processing (HPP); pasteurization; Salmonella; Lactobacillus; food safety; Nigeria; non-thermal processing

INTRODUCTION

Packaged fruit juices rank among the most widely consumed ready-to-drink beverages in sub-Saharan Africa, driven by rising urbanization, convenience, and a perception of nutritional value. Yet their physicochemical profile characteristically low pH (3.0–4.5), high water activity, and rich carbohydrate content simultaneously supports the growth of acid-tolerant pathogens and spoilage microorganisms (Daher et al., 2017). Foodborne illnesses linked to contaminated juices have been documented worldwide, caused by *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, and toxigenic *Staphylococcus aureus*, among others (Silva & Evelyn, 2023; Neggazi et al., 2024). In Nigeria

specifically, several surveys have detected total bacterial counts of 10^4 – 10^5 CFU/mL and occasional pathogen isolates in branded packaged juices (Ogodo *et al.*, 2016; Oranusi, 2012), yet systematic, brand-comparative data remain limited.

Conventional thermal pasteurization (typically 70–90°C for seconds to minutes) constitutes the primary safety intervention for juice manufacturers. Whilst effective against most vegetative pathogens, thermal treatment causes undesirable Maillard reactions, ascorbic acid degradation, colour alteration, and off-flavour formation, all of which compromise the fresh-like quality sought by modern consumers (Silva & Evelyn, 2023). High-pressure processing (HPP) — the application of isostatic pressures of 400–600 MPa for 1–6 minutes at ambient or chilled temperatures — has emerged as a superior alternative, delivering ≥ 5 –8 log reductions in vegetative pathogens whilst retaining vitamins, antioxidants, colour, and fresh aroma (Balasubramaniam *et al.*, 2015; Daher *et al.*, 2017). HPP has received regulatory endorsement from the U.S. FDA (Juice HACCP), the European Food Safety Authority (EFSA, 2022), and is commercially deployed across juice categories globally.

The present study addresses two interlinked objectives: (i) to characterize the current microbiological status of three leading Nigerian packaged juice brands through plate count methods, selective enumeration, and biochemical identification of isolates; and (ii) to contextualize the findings within the HPP literature to assess whether non-thermal intervention could close the safety gaps identified. The results are intended to inform both manufacturers and regulatory bodies (NAFDAC, SON) on the adequacy of existing processing controls and the potential value of next-generation preservation technology.

MATERIALS AND METHODS

Sample Procurement

Three commercially available packaged fruit juice brands were selected on the basis of market share and widespread retail availability in Nigeria: Exotic (Chi Exotic; mango/orange nectar), 5 Alive (blended citrus juice), and Capri-Son (Capri-Sun-style pouch drink). Three independent retail units of each brand (replicates A, B, and C) were purchased from separate outlets in a single Nigerian city on the same day, transported under refrigeration (4°C), and analyzed within 24 h of purchase.

Sterilization and Media Preparation

All glassware, Petri dishes, and reagents were sterilized by moist-heat autoclavation (121°C, 15 min). Work surfaces were disinfected with 70% (v/v) ethanol before and after each session. Seven culture media were prepared according to manufacturer instructions and autoclaved: Nutrient Agar (NA, 28 g/L) for total heterotrophic counts; MacConkey Agar (MA, 51.53 g/L) for Gram-negative enteric bacteria; Mannitol Salt Agar (MSA, 111 g/L) for staphylococci; Salmonella-Shigella Agar (SSA, 63 g/L) for enteric pathogens; Cetrinide Agar (CA, 43.3 g/L) for *Pseudomonas* spp.; Thiosulfate-Citrate-Bile-Sucrose Agar (TCBS) for *Vibrio* spp.; and de Man-Rogosa-Sharpe Agar (MRS) for lactic acid bacteria.

Enumeration and Plating

Serial ten-fold dilutions were prepared by aseptically transferring 1 mL of juice into 9 mL sterile normal saline. Dilutions were continued to 10^{-7} ; plating was performed from the 10^{-3} to 10^{-4} dilutions using the pour-plate technique (1 mL inoculum + 20 mL molten agar at $\approx 45^\circ\text{C}$). Plates were incubated at 37°C for 24 h, after which distinct colonies were counted and expressed as colony-forming units per millilitre (CFU/mL). All enumerations were performed in triplicate; means are reported.

Isolation, Gram Staining, and Biochemical Identification

Morphologically distinct colonies were subcultured on NA until pure cultures were obtained. Gram staining was performed by the standard four-step method (crystal violet, Lugol's iodine, 95% ethanol decolorization, safranin counterstain) and examined by oil-immersion light microscopy. Presumptive species identification was based on a panel of biochemical tests: oxidase (filter-paper method), catalase (3% H_2O_2 tube method), citrate utilization (Simmons' Citrate Agar, 30°C, 24 h), indole production (Kovac's reagent, peptone water, 37°C, 48 h), Kligler Iron Agar (KIA; glucose and lactose fermentation, H_2S and gas production), and motility (SIM medium stab, 35°C, 18–24 h). For *S. aureus* a slide coagulase test was also performed.

RESULTS

Heterotrophic Plate Counts

Total viable bacterial counts across all nine samples are summarized in Table 1. Counts ranged from 2.37×10^3 (Exotic A) to 3.60×10^3 CFU/mL (5 Alive B). Brand-level means were: Exotic, 2.61×10^3 CFU/mL; 5 Alive, 3.26×10^3 CFU/mL; Capri-Son, 2.80×10^3 CFU/mL. No sample exceeded 4.0×10^3 CFU/mL.

Table 1. Heterotrophic bacterial counts (colony counts per plate and mean CFU/mL) for nine juice samples across three brands.

Sample	Rep 1	Rep 2	Rep 3	Mean CFU/mL
Exotic A	23	23	25	2.37×10^3
Exotic B	28	29	26	2.77×10^3
Exotic C	27	25	29	2.70×10^3
5 Alive A	33	32	34	3.30×10^3
5 Alive B	37	35	36	3.60×10^3
5 Alive C	29	28	29	2.87×10^3
Capri-Son A	30	31	29	3.00×10^3
Capri-Son B	28	26	27	2.70×10^3
Capri-Son C	26	29	26	2.70×10^3

Selective and Differential Media Enumeration

No growth was observed on Cetrimide or TCBS agars for any sample, indicating the absence of *Pseudomonas* and *Vibrio* spp. Growth occurred on MacConkey (1.05 – 1.50×10^4 CFU/mL in Exotic and Capri-Son; 5.50 – 9.00×10^3 CFU/mL in 5 Alive B–C), MSA (2.00 – 5.00×10^3 CFU/mL), SSA (2.00 – 5.00×10^3 CFU/mL), and MRS agars (2.00 – 5.00×10^4 CFU /mL), as detailed in Table 2.

Table 2. Microbial counts (CFU/mL) on selective and differential agars.

Sample	MacConkey	MSA	SSA	MRS	CA	TCBS
Exotic A	1.23×10^4	3.00×10^3	5.00×10^3	3.00×10^4	NG	NG
Exotic B	1.50×10^4	5.00×10^3	3.00×10^3	3.10×10^4	NG	NG
Exotic C	1.40×10^4	2.00×10^3	4.00×10^3	3.30×10^4	NG	NG
5 Alive A	NG	NG	NG	2.00×10^3	NG	NG
5 Alive B	5.50×10^3	NG	NG	3.00×10^3	NG	NG
5 Alive C	9.00×10^3	NG	NG	2.00×10^3	NG	NG
Capri-Son A	1.05×10^4	4.00×10^3	3.00×10^3	5.00×10^3	NG	NG
Capri-Son B	1.10×10^4	2.00×10^3	4.00×10^3	4.00×10^3	NG	NG
Capri-Son C	1.15×10^4	5.00×10^3	2.00×10^3	3.00×10^3	NG	NG

MSA = Mannitol Salt Agar; SSA = Salmonella-Shigella Agar; MRS = de Man-Rogosa-Sharpe Agar; CA = Cetrimide Agar; TCBS = Thiosulfate-Citrate-Bile-Sucrose Agar; NG = No growth.

Isolate Identification

Biochemical profiling of the 81 recovered isolates is shown in Table 3. Eight taxa were identified. *Salmonella* spp. was isolated from Exotic and Capri-Son but not from 5 Alive; *Shigella* spp. were similarly absent from 5 Alive. *Staphylococcus aureus* was particularly prevalent in 5 Alive (7 isolates). Capri-Son yielded the highest total isolate count (32), followed by 5 Alive (27) and Exotic (22).

Table 3. Presumptive biochemical identification of bacterial isolates.

Organism	Mot	Shape	Gram	Ox	Cit	Ind	Cat	Glc	Lac	Gas	H ₂ S	Coa
<i>B. cereus</i>	+	Rod	-	+	+	-	+	+	-	-	-	-
<i>S. epidermidis</i>	-	Cocci	+	-	-	-	+	+	+	+	+	-
<i>Lactobacillus spp.</i>	-	Rod	+	-	-	-	-	+	+	-	-	-
<i>B. subtilis</i>	+	Rod	+	-	+	-	+	+	+	-	-	-
<i>Enterobacter spp.</i>	+	Rod	-	-	+	-	+	+	-	+	-	-
<i>Salmonella spp.</i>	+	Rod	-	+	+	-	+	-	-	+	-	-
<i>Shigella spp.</i>	-	Rod	-	-	-	+	+	+	+	+	-	-
<i>S. aureus</i>	-	Cocci	+	-	+	-	+	+	+	-	-	+

Mot = Motility; Ox = Oxidase; Cit = Citrate; Ind = Indole; Cat = Catalase; Glc = Glucose fermentation; Lac = Lactose fermentation; Coa = Coagulase. + positive; - negative.

Table 4. Frequency distribution of bacterial isolates by brand.

Organism	Exotic (n)	5 Alive (n)	Capri-Son (n)	Total n (%)
<i>Lactobacillus spp.</i>	5	7	8	20 (24.69)
<i>Bacillus cereus</i>	3	5	4	12 (14.81)
<i>Staphylococcus aureus</i>	2	7	3	12 (14.81)
<i>Bacillus subtilis</i>	2	3	5	10 (12.35)
<i>Staphylococcus epidermidis</i>	2	3	3	8 (9.88)
<i>Enterobacter spp.</i>	2	2	3	7 (8.64)
<i>Salmonella spp.</i>	3	0	4	7 (8.64)
<i>Shigella spp.</i>	3	0	2	5 (6.17)
Total	22	27	32	81 (100)

Values in parentheses are percentage of total isolates (n = 81).

DISCUSSION

Overall Microbial Load and Regulatory Context

The heterotrophic counts recorded here ($2.37-3.60 \times 10^3$ CFU/mL) are substantially lower than the 10^4-10^5 CFU/mL reported in previous assessments of packaged juices from South-East Nigeria (Ogodo et al., 2016) and Owerri (Onuoha et al., 2018), suggesting that the three brands evaluated maintain comparatively good hygienic control during production. Nevertheless, detectable microbial loads persist in products marketed as pasteurized, an outcome inconsistent with a rigorously validated thermal process. Nigerian regulatory standards (NAFDAC/SON) generally require absence of pathogens and low total

counts in ready-to-drink beverages; the data presented here indicate marginal compliance at best with respect to the former criterion.

Implications of Pathogen Detection

The recovery of *Salmonella* spp. (8.64%), *Shigella* spp. (6.17%), *S. aureus* (14.81%), and *B. cereus* (14.81%) from commercially packaged, pasteurized juices is the most significant finding of this study. Enteric pathogens such as *Salmonella* and *Shigella* should be entirely absent from adequately processed products; their detection implicates either suboptimal pasteurization temperatures, inadequate holding times, or post-processing recontamination during filling, capping, or storage. This is consistent with findings from comparable Nigerian studies in which Enterobacteriaceae dominated the isolate spectrum despite commercial processing claims (Oranusi, 2012; Onuoha *et al.*, 2018). The presence of *B. cereus* and *B. subtilis* (combined 27.16%) reflects the well-established heat resistance of bacterial endospores, which can withstand conventional pasteurization temperatures and subsequently germinate under favourable storage conditions a critical gap that thermal treatment alone cannot reliably address (Daher *et al.*, 2017; Rastogi, 2022).

Staphylococcus aureus, isolated at particularly high frequency from 5 Alive (seven isolates), is a robust enterotoxin producer capable of causing illness at toxin loads generated by as few as 10^5 CFU/mL. Its presence in a juice product suggests either contamination from handlers during packaging or survival through a suboptimal thermal process. Since *S. aureus* enterotoxins are heat-stable and resist conventional pasteurization even when the vegetative cells are destroyed, early microbial control through validated inactivation steps is essential (Silva & Evelyn, 2023).

The absence of *Pseudomonas* and *Vibrio* spp. is reassuring, as these organisms would indicate specific environmental or aquatic contamination pathways not evidenced here. The dominance of *Lactobacillus* spp. (24.69%) is consistent with the acidic, nutrient-rich juice matrix, in which these acid-tolerant lactic acid bacteria are the most competitive survivors of thermal stress. Although generally non-pathogenic, their prevalence at these levels contributes to product spoilage and indicates incomplete inactivation of the natural flora (Noumavo *et al.*, 2023; Ogodo *et al.*, 2016).

Fit-for-Purpose Analysis: The Case for HPP

Taken together, the microbial profile observed across these brands including low but non-zero total counts, recovery of enteric pathogens, and dominance of spore-forming taxa maps precisely onto the limitations that high-pressure processing (HPP) is designed to overcome. At commercial parameters of 500–600 MPa for 2–5 minutes, HPP consistently achieves ≥ 5 –8 log reductions of *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* in acidic juice matrices (pH <4.6), meeting or exceeding FDA Juice HACCP requirements (Usaga *et al.*, 2021; Podolak *et al.*, 2020). Because HPP operates at ambient or refrigerated temperatures, it avoids the Maillard reactions, ascorbic acid losses, and off-flavour formation associated with thermal processing, while extending refrigerated shelf life to 30–90 days (Balasubramaniam *et al.*, 2015; Liu *et al.*, 2021).

The spore-forming organisms identified here *B. cereus* and *B. subtilis* represent a key residual challenge. Endospores can survive HPP at ambient temperatures up to 800 MPa, necessitating a combined approach: HPP paired with mild heat (50–60°C) or natural antimicrobials (e.g., nisin, organic acids) can achieve the spore inactivation otherwise inaccessible by either intervention alone (Rastogi, 2022; Lou *et al.*, 2022). For the acidic matrices of the juices evaluated (pH 3–4.5), the synergy between low pH and elevated pressure further enhances inactivation of vegetative cells, making HPP particularly suited to this product category (Silva & Evelyn, 2023).

Critically, EFSA (2022) concluded that HPP at commercial parameters introduces no new microbial or chemical safety concerns relative to thermally processed equivalents. Despite higher capital costs (\approx USD 500,000–3,000,000 per unit), the expansion of tolling networks across Africa and the demonstrated per-unit operating cost of 4–10 cents/lb make HPP increasingly accessible to mid-tier manufacturers. Nigerian brands targeting premium, child-oriented, or export markets — such as those examined here — are plausible early adopters (Balasubramaniam *et al.*, 2015; SkyQuest, 2025).

Limitations

This study employed culture-based identification without molecular confirmation (e.g., 16S rRNA sequencing), and is therefore limited to presumptive taxonomic resolution. The sample size (three brands, three replicates each) is sufficient for exploratory conclusions but precludes statistical inference across the broader Nigerian market. Additionally, the study design does not include HPP challenge trials; the discussion of HPP efficacy is therefore inferential, drawing on published literature rather than direct experimental evidence.

5. CONCLUSION

This study demonstrates that three leading Nigerian packaged juice brands harbor low but non-zero microbial loads, including enteric pathogens and toxin-producing organisms unexpected in commercially pasteurized products. The data suggest that current thermal processing regimes are inadequate for consistent ≥ 5 -log pathogen elimination, whether due to suboptimal process parameters, post-processing contamination, or the inherent heat resistance of spore-forming bacteria. High-pressure processing, operating at 400–600 MPa, is mechanistically suited to close these gaps: it delivers superior vegetative pathogen inactivation, is compatible with low-pH juice matrices, and preserves the fresh-like sensory and nutritional attributes that differentiate premium packaged beverages. Regulatory bodies (NAFDAC, SON) should mandate routine pathogen batch testing and encourage HPP validation studies for Nigerian juice manufacturers. Future research should combine HPP challenge trials on local juice formulations with molecular identification methods and consumer acceptability assessment to build a comprehensive, market-relevant evidence base.

DECLARATIONS

Funding: The authors received no specific funding for this work.

Conflicts of interest: The authors declare no conflicts of interest.

Data availability: Raw colony count data are available from the corresponding author upon reasonable request.

Ethical approval: Not required; all samples were commercially purchased retail products.

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