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Impact Of Preservation Strategies On Physicochemical Stability, Microbial Safety, And Antioxidant Properties Of Indigenous Non-Alcoholic Beverages In Onitsha, Nigeria

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Abstract:
This research focused on the impact of various preservation techniques on the microbial safety, physicochemical and the antioxidant properties of the indigenous non-alcoholic beverages (soymilk and zobo) sold in Onitsha, Nigeria. Each of the Soymilk and Zobo samples were separated into twenty 330ml samples which were stored under 4-6 degrees Celsius, and the following tested preservation methods: pasteurization (65 degrees Celsius for 25 minutes) and Sodium Benzoate (100 g/L) for a period of 7 days. Analyses were done on days 1, 3, and 7. Standard microbiology techniques were used for the assessment of the microbial quality of the samples, and the subsequent identifications of the isolates were done through biochemical and molecular methods. The physicochemical characteristics including the pH, titratable acidity, and the antioxidant activity (ABTS assay), while the other characteristics including the color and smell were evaluated. Among the samples, eight (8) bacterial isolates were identified including the Bacillus cereus, and six (6) which included the Aspergillus niger. Pasteurization plus Refrigeration yielded the least of all Microbial counts for all days, when versus all other methods, including the Sodium Benzoate method and the refrigeration only method. For example, on day 1 the total counts of bacterial, yeast, and enteric for the Pasteurized Soymilk were 1.57×10^4, 1.8×10^4 and 4.8×10^3 CFU/mL, respectively. By day 3, there was a significant reduction with undetected levels of mold and enteric growth. In both beverages, Pasteurization and refrigeration-induced higher physicochemical stability with minimal changes in zobo's pH (3.15–3.14), titratable acidity, colour, and odour when compared with other treatments. Antioxidant analysis revealed that ABTS radical scavenging activity showed the highest stability in pasteurized samples during the entire storage period. Overall, the combination of pasteurization and refrigeration maintained the microbial quality, physicochemical stability, and antioxidant properties of soymilk and zobo more effectively during refrigerated storage than the other methods.

Keywords: *Preservation methods; Soymilk; Zobo; Microbial safety; Antioxidant activity*

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I. Introduction

The steady growth of Nigeria's populace drives changes to the country's food distribution and production channels with a rise in the need for safe, steady, and nutritionally sufficient food and beverage products [1]. Preservation of food is still of utmost importance in ensuring food safety to keep microbiological hazards controlled, chemically and nutritionally packed quality of food, safe from quality loss due to spoilage [2] Traditional methods like refrigeration and the use of chemical preservatives are commonplace, while innovations such as hindrance and high-end soluble processing are still emerging [3] Nevertheless, the preservation of food substances is dependent on which food is being preserved, the methods used, and the food being handled.

In Nigeria, the consumption of soymilk and zobo is commonplace, and the beverages are susceptible to spoilage because of moisture, nutrient, and poor hygienic control and preservation supplemented with the lacking standard preservation techniques. Rapid deterioration, poor quality, and limited spoilage control due to microbial contamination can all be attributed to poor control preservation techniques. A quality beverage has balanced and stable physicochemical attributes which are its pH, and appetitive characters like colour, taste, and smell, all of which are affected by change in temperature [4]

The incorporation of microbe-based contamination of drinks is a risk to public health because the source of the food-borne pathogens may include the use of contaminated raw materials, the use of non-sterilized equipment, the food handler's unclean/hygienic personal behavior, and unclean foods,). Foodborne pathogens related to drinks may include Salmonella, Campylobacter, Enteric bacteria, and yeasts and molds [5]. The World Health Organization (2023) estimated 600 million illnesses and 420,000 deaths in a year due to contaminated food [6]

Preservation methods such as refrigeration, chemical preservatives (e.g., sodium benzoate), and pasteurization are effective in limiting microbial growth. However, methods used in combination usually yield better results than those used individually.Since the primary concern in the microbial safety of beverages processed for sale is the safety of the consumers, the safety of the consumers must be ensured. The maintenance of the protective value of the beverage is necessary for the functional value and nutritional value of the beverage. While antioxidants maintain nutritional value, they also prevent oxidative damage in the cells, as they bind to and remove free radicals and reactive particles in cells [7] The use of preservation techniques such as pasteurization and refrigeration has been reported to prolong the preservation of antioxidants in plant-based beverages, increasing the stability of the products and the acceptance of the products by consumers [8]. While these beverages are common in the market, there is little information available on the preservation techniques, microbial safety, physicochemical quality, and antioxidant retention of the indigenous beverages sold in urban markets of Nigeria [9].

Thus, the research aims to study the preservation techniques on the soymilk and zobo vended in Onitsha, Nigeria, evaluating the effects of refrigeration, sodium benzoate, and pasteurization, seeking to identify the preservation techniques that enhance the safety, quality, and shelf-life of the products.

II. Materials And Methods

Study Area

The Samples were purchased from two different markets in Onitsha Anambra state; Ochanja Market and Main Market both markets in Onitsha South and Onitsha North local governments respectively. Onitsha is located within latitude 6.14131N and longitude 6.80249E. Both areas are bound by Ogbaru Local government to the south, Idemili North and Oyi to the east and Anambra East to the North.

Sample collection

Twenty Soymilk and Zobo samples contained in a sterile plastic bottles measuring 300ml each (40 samples) were purchased from vendors from Onitsha Main Market (10 zobo samples and 10 soymilk) and Ochanja Market (10 zobo samples and 10 soymilk samples). These samples were put in an ice-packed plastic container and were transported to Nnamdi Azikiwe university microbiology laboratory immediately for analyses. The samples were refrigerated throughout the period of analysis.

Isolation of Microorganisms.

Serial dilutions of each sample were prepared up to 10^{-3} in a sterile distilled water [10].

Inoculation, Incubation and Storage of the Isolates.

After the 10-fold serial dilution up 10^{-2}. An aliquot of 2ml serial sample was pipetted into the petri dishes containing MacConkey agar or Potato dextrose agar . The above media were selective media for bacteria and fungi indicator organisms respectively Both MacConkey agar and Potato dextrose agar were prepared according to manufacturer's Specifications [11]

Microbial Count

The microbial count was expressed in standard method according to cheesbrough,(2010) as follows; CFU/mL/ Volume plated x Dilution factor; where CFU/Ml means coliform forming unit per milliliter and dilution factor is 10.

Morphological Characterization.

Morphological characterization was carried out using standard methods [12]

Gram Staining of Bacterial Isolates

Gram staining of bacterial isolates were carried out using the standard differential technique as it was described by Cheesbrough

Other Bacterial Biochemical Characterization

The following confirmatory biochemical test was carried out on the bacterial isolates using a standard method [13]. The tests were as follows: the results were recorded in real time; Indole Test, Methyl Red Test, Motility Test, Voges-Proskauer Test, Catalase Test, Citrate Test, Coagulase Test, Urease Test, Oxidase Test and Sugar Fermentation Test,

Molecular Characterization of Bacterial and Fungal Isolates

Molecular characterization of isolated bacteria and fungal isolates were based on 16srRNA conserved gene sequencing using Universal bacterial primes .The targeted were amplified using the conventional PCR method and the size of the amplified fragments were confirmed by running the final product by 1% gel electrophoresis. The amplified sequencing fragments were sent for and the retrieved nucleotide sequence were phylogenetically studied using MEGA software (MEGA-11). Bacterial isolates were further verified at the species level by BLAST search using GENBank NCBI(National Centre for Bacteriology Information [14].

Morphological Characterization of Fungal Isolates

The morphological characterization/identification of fungal isolates was carried following standard method as described by [15].

Biochemical test for fungal isolates

Biochemical tests for fungal isolates were carried out using standard methods as described by [16]. The tests carried out were: nitrate reduction test, lactophenol cotton blue staining test and sugar fermentation test.

The Physicochemical Analysis of Zobo and Soymilk

Physiochemical properties of soymilk are pH, total dissolved solids, specific gravity, titratable acidity, taste, smell, color and texture. All physicochemical tests were carried out according to specifications by [17]

PH

This is logarithmic scale used to specify the degree of acidity, neutrality and basicity of a solution. This is measured with *pH* meter. Soymilk and zobo samples measuring 300ml each was poured into a sterilized beaker and *pH* meter was inserted into the solution and the records were taken. The *pH* values were taken for 28 days duration at 7days intervals. All samples were being refrigerated throughout the research period.

300ml of soymilk and zobo samples were used each day for the test. Note that the *pH* meter was calibrated with deionized water to ensure accuracy of result. AS ONE KRSE *pH* meter was used.

Specific Gravity

This is used to measure the specific dissolved solid in a solution (soymilk and zobo samples). It is measured with a hydrometer. The soymilk and zobo samples were poured into a 1000ml of measuring cylinder, then the hydrometer was inserted into the liquid and the reading was taken. Calibration of the hydrometer was done with distilled water. Cole Parmer hydrometer was used.

Brix/Total Dissolved Sugar

This measures the total amount of sugar in a solution. It is measured with a calibrated refractometer. The refractometer must be calibrated with deionized water. An aliquot volume was collected from a sample of soymilk and Zobo with the use of sterilized miniature pipette into the well cleaned glass surface of a calibrated refractometer. This test was done where there is no intense light source. Atago make of refractometer was used

Titratable Acidity

The Total Titratable Acidity (TTA), calculated as lactic acid was determined by titrating 0.1M sodium hydroxide against five milliliters of the Zobo and soymilk samples using phenolphthalein as an indicator 2.**9.5**

Temperature

The temperature of the samples were measured using a calibrated laboratory glass thermometer. Testo thermometer was used.

Sensory Evaluation

Within the 7 days duration of the study, physical appearances, taste and odour of the samples of soymilk and zobo was examined and recorded on everyday basi**s.**

Antioxidant activities of Soymilk and Zobo

The anti-oxidant activities of soymilk and Zobo were analyzed thus using Varian cary 400 Scan UV visible spectrophotometer based on AOAC 2019 guidelines [18]. The model of spectrophotometer used was thermo scientific Genesys 180 UV-Vis. The control of the experiment was blank solvent(Water) without the samples. The principle, the calculation and full methodology is located in appendix of this work. The following are the assays and the methods of the tests.

Detection of ABTS free radical scavenging ability

Add 1 mL soymilk and zobo in two different test tubes to 2.5 mL ABTS detection solution, shake and mix thoroughly, and then store in dark at room temperature for 30 min. Finally, the absorbance value was measured at 734 nm and the free radical scavenging rate of ABTS was calculated.

Detection of DPPH radical scavenging ability

The AOAC method for detecting DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, as per AOAC, 2019 guidelines. The samples were added to the DPPH solution and were incubated for 30 minutes at room temperature. The absorbance was calculated at 517nm using a spectrophotometer. The DPPH radical scavenging activity was calculated as a percentage of the control (without sample).The control was a blank solvent without sample is as follows:

Detecting of FRAP scavenging ability

The AOAC method for detecting FRAP (Ferric Reducing Ability of Plasma) scavenging ability, as per the 2019 guidelines, is as follows: The method measures the ability of a sample (Zobo &Soymilk) to reduce ferric ions (Fe3+) to ferrous ions (Fe2+). The unit of measurement is mmol Fe^{2+}/L.

Preservation methods

Refrigeration

The samples were refrigerated throughout the whole experiment period within the temperature range of 4-6 degrees Celsius. [19] The refrigeration lasted for 7 days. According to the FDA, refrigeration of beverages should follow these guidelines; the beverages should be stored at 40°F (4°C) or below.

Addition of Preservatives

Sodium sorbate and sodium benzoate were added at 100g/1000ml of Soymilk and 100g /1000ml of Zobo samples.

Pasteurization

Soymilk and zobo samples were pasteurized according to the method by [20].The following steps were observed; pour water into bottom part of the double boiler until it is half full. Fill the top part of the double boiler with less than 16 cups (about 5000ml) of soymilk samples and zobo samples respectively at different times . The burner was turned on a little at a time to heat up the samples slowly, use the cooking thermometer to monitor the temperature of the samples, don't allow the thermometer to rest on the bottom or sides of the boiler .The samples were stirred as often as it is heating up. Heat the samples to at least 63 degree celcius for 30 minutes. The samples were cooled and put on the top part of the double boiler in the ice water bath to cool them fast. The samples were stirred often to cool them faster until it reaches 20 degree.

Post Preservation Microbial Load Assessment, Physicochemical Properties and Antioxidant properties Analysis of the soymilk and zobo Samples.

The soymilk and zobo samples microbial load, physiochemical properties and antioxidant properties were reassessed after refrigeration, Pasteurization and sodium sorbate addition in order to determine the effects of the different preservation treatments on the samples.

Statistical Analysis

Analysis of variance (One way ANOVA) was used to determine the effects of different preservation methods on physiochemical properties, microbial load and antioxidant activities of Soymilk and zobo using IBM SPSS version 20 Turkey Urachal.

III. Results

Microbial Counts and Analysis of Refrigerated Soymilk Sample

According to table 5, the refrigerated soymilk sample (S3RF) recorded, on Day 1**,** a total bacterial count of 8.7×10^3 CFU/mL ± 6.5×10^2 CFU/mL**,** a total yeast count of 2.6×10^3 CFU/mL ± 1.9×10^2 CFU/mL**,** a total mold count of 1.0×10^2 CFU/mL ± 2.0×10^1 CFU/mL**,** and an enteric bacterial count of 1.9×10^3CFU/mL ± 2.3×10^2CFU/ml.

These results similarly point to contamination from poor handling or unsanitary processing conditions.

By Day 3**,** the total bacterial count declined to 3.0×10^3 CFU/mL ± 1.78×10^2 CFU/mL**,** yeast count to 2.9×10^3 CFU/mL ± 3.2×10^2 CFU/mL**,** with no mold growth detected. The enteric bacterial count dropped to 4.4×10^2 CFU/mL ± 2.2×10^2 CFU/mL**.**

On Day 7, microbial resurgence occurred, with total bacterial count increasing to 1.98×10^4 CFU/mL ± 1.2×10^3 CFU/mL**,** yeast count to 7.0×10^3 CFU/mL ± 1.1×10^2 CFU/mL**,** while molds remained undetected. The enteric bacterial count rose to 3.8×10^3 CFU/mL ± 2.1×10^2 CFU/mL**.**

A comparative assessment shows that the refrigerated soymilk (S3RF**)** exhibited slightly lower bacterial growth **(**1.98×10^4 CFU/mL) than the pasteurized sample (S1P) **(**2.47×10^4 CFU/mL) after seven days, indicating that cold storage enhanced microbial stability. A similar pattern was observed in Sample 4 (S4RF)**.**

Microbial Count of Sodium Benzoate Treated Soymilk Samples

Table 5 revealed that the sodium benzoate treated soymilk sample 5 (S5SB) recorded on the 1st day (day 1) a total bacterial count of 3.64×10^4CFU/mL±2.3×110CFU/mL, total yeast count of 4.4×10^3CFU /mL±1.4×10^2mL, no mold growth and no enteric bacteria. Day 3 microbial count recorded no bacterial growth, total yeast count 3.0×10^4CFU/mL±1.0×10^2CFU/mL, no mold growth and no enteric bacterial growth. The general phenomenom in the results obtained in the research is the general decline in microbial load on the third day. This shows that the cells of microorganisms that withstood pasteurization temperature takes time to recuperate to begin proliferation. This goes to show that pasteurization alone does not kill the spores of microorganisms. There were resurgences total bacterial count of 4.5×10^3CFU/mL±1.1×10^2CFU/mL, total yeast count of 5.7 × 10 ³ CFU / Ml ± 1.7×10^2CFU/mL , total mold count of 3.0×10^3CFU/mL±1×10^2CFU/mL There was a total enteric bacterial count of 5.0×10^3CFU/mL±1×10^2CFU/mL. This same trend was observed for S10SB.

Microbial Count of Pasteurized Zobo Samples (PZ1 and PZ2)

Table 6 reveals that the pasteurized zobo samples (PZ1 and PZ2) recorded a total bacterial count of 4.98×10^3 CFU/mL ± 2.7×10^2 CFU/mL**,** a total yeast count of 1.5×10^3 CFU/mL ± 1.0×10^2 CFU/mL, with no mold or enteric bacterial growth observed on Day 1 (baseline). On Day 3**,** there was no detectable microbial growth for all parameters (bacteria, yeast, mold**,** and enteric bacteria). However, by Day 7, a resurgence in microbial load was observed: total bacterial count increased to 8.4×10^3 CFU/mL ± 5.3×10^2 CFU/mL**,** yeast count to 5.6×10^3 CFU/mL ± 2.4×10^2 CFU/mL**,** mold count reached 4.2×10^2 CFU/mL, and enteric bacterial count rose to 4.4×10^2 CFU/mL ± 5×10^1 CFU/mL**.** Both PZ1 and PZ2 exhibited similar microbial growth patterns throughout the observation period.

Microbial Count of Refrigerated Zobo Samples (Z3RF, Z4RF, and Z8RF)

Table 6 shows that the refrigerated zobo sample 3 (Z3RF) had a total bacterial count of 4.0×10^4 CFU/mL ± 9.2×10^2 CFU/mL on Day 1**,** with no yeast or mold growth, and an enteric bacterial count of 5.8×10^3 CFU/mL ± 1.3×10^2 CFU/mL. On Day 3**,** the total bacterial count reduced to 3.0×10^3 CFU/mL ± 1.2×10^2 CFU/mL**,** while yeast appeared **at** 3.0×10^3 CFU/mL ± 1.0×10^2 CFU/mL; no mold or enteric bacterial growth was detected. By Day 7, microbial resurgence occurred: total bacteria increased to 1.07×10^4 CFU/mL ± 5.5×10^2 CFU/mL**,** total yeast count rose to 7.7×10^3 CFU/mL ± 1.2×10^2 CFU/mL**,** mold count reached 8.2×10^2 CFU/mL ± 1.4×10^2 CFU/mL, and enteric bacteria to 1.7×10^3 CFU/mL ± 1.1×10^2 CFU/mL.

For sample Z4RF**,** Day 1 total bacterial count was 3.4×10^4 CFU/mL ± 2.2×10^2 CFU/mL**,** total yeast count 2.05×10^4 CFU/mL ± 1.0×10^2 CFU/mL, mold count 6.1×10^2 CFU/mL ± 1.9×10^1 CFU/mL, and enteric bacteria 4.7×10^3 CFU/mL ± 2.1×10^2 CFU/mL**.** On Day 3**,** microbial load decreased to 2.4×10^3 CFU/mL ± 1.1×10^2 CFU/mL for bacteria and 4.0×10^3 CFU/mL ± 2.2×10^1 CFU/mL for yeast, with no mold or enteric bacteria detected. By Day 7**,** total bacterial count increased again to 7.9×10^3 CFU/mL ± 2.3×10^2 CFU/mL, total yeast to 6.2×10^3 CFU/mL ± 2.1×10^2 CFU/mL, and enteric bacteria to 2.3×10^3 CFU/mL ± 1.1×10^2 CFU/mL, while mold remained undetected.

The refrigerated zobo sample 8 **(**Z8RF**)** exhibited a comparable trend, confirming that although refrigeration slows enzymatic activity and microbial proliferation, it does not completely inhibit bacterial and fungal growth, hence the observed resurgence after prolonged storage.

Microbial Count of Sodium Benzoate–Treated Zobo Samples (Z9SB and Z10SB)

As shown in Table 6, sample Z9SB recorded a total bacterial count of 2.1×10^3 CFU/mL ± 1.3×10^1 CFU/mL on Day 1, with no yeast or mold growth and an enteric bacterial count of 1.1×10^3 CFU/mL ± 1.0×10^2 CFU/mL. On Day 3, the total bacterial count decreased to 1.1×10^2 CFU/mL ± 1.5×10^2 CFU/mL, mold count rose slightly to 2.0×10^3 CFU/mL ± 1.1×10^2 CFU/mL**,** while yeast and enteric bacteria were absent. By Day 7, total bacterial load increased markedly to 9.4×10^3 CFU/mL ± 2.3×10^2 CFU/mL, with total yeast count 5.0×10^3 CFU/mL ± 1.9×10^2 CFU/mL**,** mold count 5.7×10^3 CFU/mL ± 1.1×10^2 CFU/mL**,** and enteric bacteria 5.3×10^3 CFU/mL ± 1.2×10^2 CFU/mL.

Sample Z10SB followed a similar trend except that no microbial growth (bacteria, yeast, mold, or enteric bacteria) was recorded on Day 3**,** indicating a temporary inhibitory effect of sodium benzoate during mid-storage.

Table 5. Mean Bacterial, Fungal Count Across 7 days in Soymilk Samples

Samples	**Days**	Total Bacterial Count (CFU/mL)	Total yeast count (CFU/mL)	Total mold count (CFU/mL)	Total enteric bacteria count (CFU/mL)
S1P	Day 1	$1.57x10^4 \pm 1.03x10^3$	$1.8 x10^4 \pm 1.1 x10^2$	NG	$4.8 x10^3 \pm 3.5x10^2$
	Day 3	$1.3x10^3 \pm 9.8x10^2$(tftc)	NG	NG	NG
	Day 7	$2.47x10^4 \pm 1.98x10^2$	$9.7x10^3 \pm 2.3x10^2$	NG	$4.5x10^3 \pm 2.1x10^2$
S2P	Day 1	$1.01x10^3 \pm 7.8x10^2$	NG	NG	$5.2x10^2 \pm 1.8x10^2$ tftc
	Day 3	NG	NG	NG	NG
	Day 7	$1.92x10^4 \pm 1.07x10^3$	$1.8x10^4 \pm 2.1x10^2$	$3.4x10^3 \pm 2.8x10^2$	$3.7x10^3 \pm 2.3x10^2$
S3RF	Day 1	$8.7 x10^3 \pm 6.5x 10^2$	$2.6x10^3 \pm 1.9x10^2$ tftc	$1.0 x10^2 \pm 2x10^1$ tftc	$1.9 x10^3 \pm 1.2x10^2$ tftc
	Day 3	$1.93x10^3 \pm 1.78x10^2$ (tftc)	$2.9x10^3 \pm 3.2x10^2$ tftc	NG	$4.4x10^2 \pm 2.2x10^1$
	Day 7	$1.37x10^4 \pm 1.03x10^3$	$7.0x10^3 \pm 1.1x10^2$	NG	$3.8x10^3 \pm 2.1x10^1$
S4RF	Day 1	$6.7 x10^3 \pm 3.3x10^2$	$1.72 x10^4 \pm 1.38x10^2$	$3.2 x10^2 \pm 1.8x10^1$ tftc	NG
	Day 3	NG	$3.2x10^3 \pm 1.8x10^2$ tftc	$1.0x10^2 \pm 2.2x10^1$	NG
	Day 7	$1.98x10^4 \pm 1.22x10^3$	$1.43x10^3 \pm 2.2x10^2$	$3.7x10^3 \pm 1.4x10^2$	$7.0x10^3 \pm 1.7x10^2$
	Day 7	$7.89x10^3 \pm 2.3x10^2$	$6.2x10^3 \pm 2.1x10^2$	NG	$2.3x10^3 \pm 1.1x10^2$ tftc
S5SB	Day 1	$3.6x10^4 \pm 2.0x10^2$	$4.4 x10^3 \pm 1.4x10^2$	NG	NG
	Day 3	$3.0\times10^4 \pm 1.0\times10^2$	$3.3x10^3 \pm 1.0.2x10^2$	$2.4\times10^3 \pm 1.0\times10^2$	NG
	Day 7	$4.5x10^3 \pm 1.1x10^2$	$5.7x10^3 \pm 1.7x10^2$	$3.0x10^3 \pm 1x10^2$ tftc	$5.0x10^3 \pm 1x10^2$
S6SB	Day 1	$7.6 x10^3 \pm 1.9x10^2$	NG	NG	NG
	Day 3	NG	$1.6x10^3 \pm 1.5x10^2$ tftc	NG	NG
	Day 7	$5.0x10^3 \pm 1.8x10^2$	$6.4x10^3 \pm 1.4x10^2$	$3.4x10^3 \pm 1.1x10^2$	$5.3x10^3 \pm 1.4x10^2$
S7SB	Day 1	$4.3 x10^3 \pm 1.1x10^2$	$3.1x10^3 \pm 1.3x10^2$	$3.0 x10^2 \pm 1.7x10^1$	NG
	Day 3	$6.0x10^3 \pm 2.3x10^2$(tftc)	$1.78x10^3 \pm 1.2x10^2$	NG	$3.2x10^3 \pm 1.1x10^2$ tftc
	Day 7	$5.4x10^3 \pm 2.7x10^2$	$5.0x10^3 \pm 3.2x10^2$	$4.1x10^3 \pm 2.1x10^2$	$4.7x10^3 \pm 1.8x10^2$
S8SB	Day 1	NG	NG	NG	NG
	Day 3	NG	$9.1x10^3 \pm 2.7x10^2$ tftc	NG	$3.2x10^3 \pm 2.1x10^2$ tftc
	Day 7	$4.6x10^3 \pm 3.2x10^2$	$5.8x10^3 \pm 2x10^2$	$5.2x10^3 \pm 1.8x10^2$	$6.0x10^3 \pm 1.5x10^2$
S9SB	Day 1	$5.3x10^2 \pm 1.5x10^2$(tftc	NG	NG	NG
	Day 3	NG	$1.2x10^3 \pm 2.4x10^2$	NG	NG
	Day 7	$7.5x10^3 \pm 3.2x10^2$	$6.7x10^3 \pm 2.8x10^2$	$5.4x10^3 \pm 1.6x10^2$	$5.4x10^3 \pm 1.7x10^2$
S10SB	Day 1	NG	$9.2 x10^2 \pm 1.8x10^1$	NG	NG
	Day 3	$1.89x10^3 \pm 1.1x10^2$	$1.38x10^3 \pm 3x10^2$	NG	NG
	Day 7	$7.3x10^3 \pm 6.7x10^2$	$7.3x10^3 \pm 1.9x10^2$	$4.5x10^3 \pm 2.7x10^2$	$5.8x10^3 \pm 1.9x10^2$

N.B: p-values obtained using one way ANOVA; $p<0.05$ considered significant

The baseline (day 1 samples and their parameters) are the reference samples/control

Keys:

S1P=Pasteurized soymilk sample 1

S2P= Pasteurized soymilk sample2

S3RF= Refrigerated soymilk sample S4RF = Refrigerated soymilk sample 4 S5SB= sodium benzoate treated soymilk sample 5S6SB= sodium benzoate treated soymilk sample 6

Same naming pattern for S7SB,S8SB, S9SB and S10

Table 6. Mean Bacterial, and fungal counts across 7 days in soymilk samples

Samples	Days	Total Bacterial Count (CFU/mL)	Total yeast count (CFU/mL)	Total mold count (CFU/mL)	Total enteric bacteria count (CFU/mL)
Z1P					
	Day 1	$4.98 x10^3 \pm 2.7x10^2$	$1.5 x10^3 \pm 1x10^2$	NG	NG

	Day 3	NG	NG	NG	NG
	Day 7	$8.4x10^3 \pm 5.3x10^2$	$5.6x10^3 \pm 2.4x10^2$	$4.2x10^2 \pm 5x10^1$(tftc)	$4.4x10^3 \pm 2.3x10^2$
Z2P					
	Day 1	$2.38\ x10^4 \pm 1.07x10^3$	$3.4\ x10^2 \pm 1.9x10^1$(tftc)	NG	$3.4x10^3 \pm 1.3x10^2$
	Day 3	NG	NG	NG	NG
	Day 7	$9.0x10^3 \pm 4.2x10^2$	$6.4x10^3 \pm 1.6x10^2$	NG	$3.7x10^3 \pm 2.1x10^2$
Z3RF					
	Day 1	$4.0x10^4 \pm 9.2x10^2$	$5.8\times10^3\pm1.3\times10^2$	NG	$5.8x10^3 \pm 1.3x10^2$
	Day 3	$3.0\times10^4 \pm 1.2x10^2$	$3.0\times10^3\pm1.0\times10^2$	NG	$3.0\times10^3\pm1.0\times10^2$
	Day 7	$1.07x10^4 \pm 5.5x10^2$	$7.7x10^3 \pm 1.2x10^2$	$8.2x10^2 \pm 1.4x10^2$(tftc)	$1.7x10^3 \pm 1.1x10^2$(tftc)
Z4RF					
	Day 1	$3.4\ x10^4 \pm 2.2x10^2$	$2.05x10^4 \pm 1x10^2$	$6.1\ x10^2 \pm 1.9x10^1$	$4.7x10^3 \pm 2.1x10^2$
	Day 3	$2.4x10^3 \pm 1.1x10^2$(tftc)	$4.0x10^3 \pm 2.2x10^2$	NG	$2.4x10^2 \pm 1.2x10^1$(tftc)
	Day 7	$7.9x10^3 \pm 2.3x10^2$	$6.2x10^3 \pm 2.1x10^2$	NG	$2.3x10^3 \pm 1.1x10^2$(tftc)
Z5P					
	Day 1	$1.25\ x10^4 \pm 2.3x10^2$	NG	NG	NG
	Day 3	NG	NG	$3.4x10^3 \pm 1.3x10^2$	$3.8x10^3 \pm 1.2x10^2$
	Day 7	$8.25x10^3 \pm 6.8x10^2$	$5.8x10^3 \pm 2.1x10^2$	NG	$2.4x10^3 \pm 1.6x10$(tftc)
Z7RF					
	Day 1	$3.5\ x10^3 \pm 2.7x10^2$	NG	NG	NG
	Day 3	$1.4x10^3 \pm 1.2x10^2$	NG	NG	NG
	Day 7	$1.3x10^4 \pm 1.2x10^2$	$9.4x10^3 \pm 2.8x10^2$	$3.6x10^3 \pm 2.4x10^2$	$1.7x10^3 \pm 1.8x10^2$(tftc)
Z8RF					
	Day 1	$7.8\ x10^3 \pm 3.7x10^2$	NG	NG	$4.2\times10^3\pm1.0\times10^2$
	Day 3	NG	$1.4x10^3 \pm 2.8x10^2$(tftc)	NG	$2.1x10^3 \pm 1.7x10^2$
	Day 7	$1.4x10^5 \pm 2.3x10^3$	$1.28x10^4 \pm 1.2x10^2$	$2.8x10^3 \pm 1.3x10^2$	$4.6x10^3 \pm 3.7x10^2$
Z9SB					
	Day 1	$2.10\times10^4\pm1.3\times10^2$	NG	NG	$1.10\times10^4\pm1\times10^1$
	Day 3	$8.7x10^2 \pm 1.5x10^1$	NG	$2x10^3$	NG
	Day 7	$9.4x10^3 \pm 2.3x10^2$	$5.0x10^3 \pm 1.9x10^2$	$5.7x10^3 \pm 1.1x10^2$	$5.3x10^3 \pm 1.2x10^2$
Z10SB					
	Day 1	$1.58\ x10^4 \pm 1.23x10^2$	$2.5\ x10^3 \pm 1.0x10^2$(tftc)	NG	$6.4\ x10^3 \pm 1.8x10^2$
	Day 3	NG	NG	NG	NG
	Day 7	$8.76x10^3 \pm 7.8x10^2$	$5.8x10^3 \pm 2.3x10^2$	$5.5x10^3 \pm 2.1x10^2$	$5.8x10^3\pm 1.7x10^2$

N.B: the naming of these samples did not follow numerical order rather numbers were assigned to the samples for identification.

;Keys:

Z1P= Pasteurized zobo sample 1

ZP2= Pasteurized zobo sample 2

Z3RF= Refrigerated Zobo Sample 3

Z4RF=Refrigerated zobo samples 4

Z5P=Pasteurized zobo sample 5

Z7RF= Refrigerated zobo sample 7

Z8RF= Refrigerated zobo sample 8

Z9SB and Z10SB= Sodium benzoate treated zobo sample 9 and sodium benzoate treated zobo sample 10

Morphological Biochemical Characterization of Bacterial Isolates

Table 7 presents the morphological and biochemical characteristics of bacterial isolates obtained from the soymilk and zobo samples. The isolates exhibited a diversity of morphological features, encompassing both Gram-positive and Gram-negative bacteria, each demonstrating distinct biochemical profiles.

The biochemical tests confirmed the presence of spoilage-associated and potentially pathogenic microorganisms, indicating microbial contamination of varying origins. Notably, the detection of enteric bacteria such as *Salmonella typhimurium* and *Escherichia coli* highlights potential public health risks arising from inadequate processing**,** poor hygiene**,** or improper storage conditions. These organisms, along with other identified bacteria, may contribute to quality deterioration**,** off-flavor development, and reduced shelf stability of the beverages, underscoring the importance of effective preservation and strict sanitary practices during production and handling.

Fungal Isolates and Their Characteristics

Table 8 presents the fungal isolates and their characteristics**,** providing further evidence of microbial degradation observed in the soymilk and zobo samples. The identified fungal species included *Aspergillus niger*, *Penicillium* spp., and *Candida* spp.—all of which are well-documented spoilage organisms known to adversely affect the flavor, aroma, and overall sensory quality of beverages.

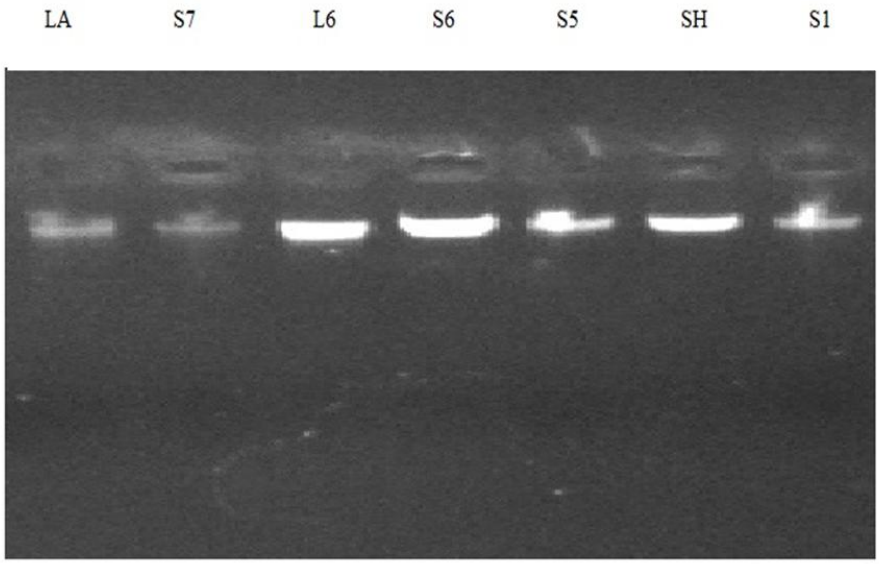
Aspergillus niger was identified as **a** major contaminant, particularly in the zobo samples. This species is of significant concern due to its ability to produce aflatoxins, which are **toxic** and carcinogenic secondary metabolites. *Penicillium* spp., also detected in multiple samples, are commonly associated with moldy spoilage and typically indicate poor storage or environmental conditions during or after production. *Candida* spp. are yeast-like fungi often linked to fermentative spoilage, leading to gas production and undesirable flavor development.

The occurrence of these fungi, especially toxigenic species such as *Aspergillus niger*, underscores the need for strict microbial control**,** hygienic handling, and the use of appropriate preservatives to prevent mycotoxin formation in ready-to-drink beverages. Fungal proliferation was most pronounced in unpasteurized samples, confirming that pasteurization significantly suppresses fungal growth and extends product shelf life.

Molecular Characterization of Bacterial and Fungal Isolates.

Micro-organisms characterized via molecular methods (short Gun and Sanger Sequencing) at fungi primer ITS and bacteria at 16sRNA sequencing. The organisms characterized were designated as:

1. isolate S1 has 99.79% pairwise similarity with *Salmonella enterica* subsp. enterica serovar Typhimurium strain with accession number; MN634487.1, and
2. isolate SH has 95.69% pairwise similarity with *Aspergillus niger* isolate with accession number:CP074650.1.



Gel image showing high molecular weight DNA extracted from the isolates

Physicochemical analysis of pasteurized Zobo Samples (PZ1 and PZ6) see table 9

The *pH* result of pasteurized zobo sample one (PZ1) remained almost unchanged from 1st day to the 3rd day (3.15-3.14) showing that no active microbial metabolic activity went on. The reason is because, heat denatured microbial enzymes and reduces viable counts leading to delay in sample spoilage. The 7th day analysis showed that the *pH* value declined from 3.14 to 2.48 showing a significant metabolic activity and consequent production of organic acids which decreased the *pH* level of the zobo sample. This is confirmed by the decrease in total devolved solids in the zobo sample (1800-1760) confirming a significant metabolism by microorganisms on the 7th day. The same trend was recorded in PZ6.

Physicochemical analysis of Refrigerated Zobo samples RZ2 and RZ3 see table 9

The *pH* value of RZ2 was 3.13 on the 1st day which declined to 2.94 on the 3rd day and further declined to 2.86 showing that combination of pasteurization and refrigeration proved more active in preserving the zobo samples than refrigeration alone. The downward change in *pH* level of the refrigerated zobo sample showed that there was metabolic activity of microorganisms which must have led to acid production .This was confirmed by a corresponding decline in the total dissolved solids of the sample which got declined from 1800 on the first day to 1733.33±57.73 on the 7th day. The titratable acidity also increased from 0.18- 0.20±0.01. The Same trend was observed in RZ3.

Physicochemical Analysis of Sodium Benzoate Treated Zobo samples SBZ4 and SB See table 9

Sodium benzoate treated zobo samples showed a *pH* value of 2.95 on the first day(day1) which declined to 2.80 on the 3rd day with a corresponding decrease in the total dissolved solids indicating commencement of fermentation by microorganisms. The sodium benzoate acts as a weak acid preservative inhibiting growth of microbes. The 7th day analysis showed a further decline in the *pH* level of the zobo which brought the zobo *pH* to 2.65 with a further reduction in total dissolved solid value. .This same trend was observed in SBZ5. This sharply marked that pasteurization+refrigeration performed best in the preservation of zobo samples than refrigeration and sodium benzoate treatment. However it was also discovered from the table that sodium benzoate treatment+refrigeration combination do better in preserving the zobo samples than refrigeration alone.

Physicochemical Analysis of Pasteurized Soymilk samples (PS4 and PS5)

The *pH* value of the pasteurized soymilk sample 4 (PS4) recorded 5.46 on the first day and remained relatively constant to about 5.45 on the third day and later declined to 4.48 on the 7th day .The trend showed that fermentation started properly on the 7th day leading to a significant decline in *pH* value. This showed that preservation of soymilk by pasteurization was more active on the 3rd day than on the 7th day. A similar trend occurred in PS5 where the *pH* of the sample on the first day was 5.46 and remained constant on the 3rd day and then declined to 5.0 on the 7th day. This showed a complete prevention of growth and metabolic activity through the heat treatment of pasteurization and later refrigeration which actually did not eliminate totally the microorganisms since it was obvious that spores of the organisms later got revived after 7 days. This gives a health threat indicating a huge contamination of the product from poor handling, vessel or equipment used in the preparation and general poor hygiene of the production processes

Physicochemical Analysis of Refrigerated soymilk samples (RS3 and RS6) see table 10

The recorded *pH* value for RS3 on the first day was 5.63 and declined to 5.50 on the 3rd day and later decreased to 4.60 on the 7th day. The same trend was observed in RS6 (refrigerated soymilk sample 6). The result showed that refrigeration alone is just able to sedate the microorganisms and not total elimination.

Physiochemical Analysis of Sodium Benzoate Treated Soymilk samples (SBS1 and SBS1) see table 10

The acidic level of the sodium benzoate treated soymilk sample SBS2 was 5.34 on the 1st day and slightly declined to 5.30 on the third day. This showed that sodium benzoate stopped the individual microorganisms from metabolizing effectively the nutrients in the sample but was not able to eliminate the spores completely resulting to a further decline in *pH* value of the sample on the 7th day. This decline corresponds with the decrease in total dissolved solids of the sample on the 7th day.

Table 7. Morphological and Biochemical Characterization of Bacterial Isolates from Soymilk and zobo Samples

Isolate	Colony Morphology (Form/Colour/Surface)	Gram Reaction	Cell Shape	Key Biochemical Features (Summary)	Probable Identity
1	Circular, shiny, cream, entire, raised, transparent	–	Rod	Catalase+, Motile+, Indole–, MR+, VP+, Citrate+, sugar fermentation+, Oxidase–, Urease+	*Proteus mirabilis*
2	Irregular, shiny, cream, entire, raised, opaque	–	Rod	Catalase+, non-motile, Indole–, MR+, VP–, Citrate+, sugar fermentation+, Oxidase–, Urease+	*Klebsiella pneumoniae*
3	Circular, mucoid, yellowish, raised, opaque	+	Cocci	Catalase+, Motile+, Indole–, MR+, VP–, mixed sugar reactions, Oxidase–, Urease+	*Staphylococcus aureus*
4	Circular, mucoid, whitish, fimbriate, raised, opaque	+	Rod	Catalase+, Motile+, Indole–, MR–, VP+, Citrate+, strong sugar fermentation, Oxidase+, variable urease	*Bacillus* spp.
5	Circular, mucoid, greyish/colourless, convex, translucent	–	Rod	Catalase+, non-motile, variable indole, MR+, VP–, weak sugar fermentation, Oxidase–, Urease–	*Shigella flexneri*
6	Circular, glistening, white, convex, moist	–	Rod	Catalase+, Motile+, Indole–, MR–, VP+, Citrate+, strong sugar fermentation, Oxidase+, Urease–	*Enterobacter aerogenes*
7	Circular, mucoid, greyish-white, lobate, low convex	–	Rod	Catalase+, Motile+, Indole–, MR+, VP–, Citrate+, selective sugar fermentation, Oxidase–, Urease–	*Salmonella typhimurium*
8	Circular, mucoid, whitish, convex, translucent	–	Rod	Catalase+, Motile+, Indole+, MR+, VP–, Citrate–, mixed sugar reactions, Oxidase–, Urease–	*Escherichia coli*

Key
+= positive
-=negative
Ag= agglutination
A= no agglutination
Cat= catalase test, ind=indole test, mot= motility test, glu= glucose test, cit=citrase test, suc=sucrose test, mal=maltose test, ur=urease test, fruc=fructose test, VP= voges proskauer Test MR= methyl red test
Var= indole variable

Table 8. Morphological and Microscopic Characteristics of Fungal Isolates

Macroscopy (Colony Features)	**Microscopic Characteristics**	**Sample Source**	**Probable Identity**
Compact white to yellow colonies with black conidial heads on PDA (48 h, 37°C)	Large globose, dark-brown conidial heads; smooth hyaline stipes; biseriate phialides; rough-walled globose conidia	Soymilk, Zobo	*Aspergillus niger*
Granular, flat yellow to yellow-green colonies with radial grooves on SDA (48 h, 37°C)	Radiate to columnar conidial heads; biseriate to uniseriate phialides; roughened stipes; echinulate globose conidia	Soymilk, Zobo	*Aspergillus flavus*
Fluffy yellowish-green colonies with bluish tint on SDA (48 h, 25°C)	Branched penicillate conidiophores; verticillate phialides; catenulate globose conidia	Zobo	*Penicillium* spp.
Smooth white to cream colonies on SDA (72 h, 37°C)	Ellipsoidal budding blastoconidia; no pseudohyphae; spherical asci with ascospores	Zobo, Soymilk	*Candida* spp.
Fast-growing floccose grey-brown colonies on PDA (72 h, 30°C)	Sympodially branched hyphae; spherical sporangia; ellipsoidal sporangiospores; brown zygospores	Zobo	*Mucor* spp.
Fast-growing white to cream aerial mycelium with yellow pigment on SDA (4 days, 30°C)	Hyaline conidiophores; fusiform, curved macroconidia with pedicellate foot cells	Zobo, Soymilk	*Fusarium* spp.

N.B: the naming of these samples did not follow numerical order rather numbers were assigned to the samples for identification.
Keys:
PZ1= Pasteurized zobo sample 1
RZ2= Refrigerated zobo sample 2
RZ3= Refrigerated zobo sample 3
SZ4= Sodium benzoate treated zobo sample 4
SZ5=Sodium benzoate treated zobo sample5
PZ6=Pasteurized zobo sample 6

Table 9. Mean values of analyzed Physiochemical Properties of Pasteurized, Refrigerated and Sodium Benzoate treated zobo samples across 7 day

Samples	*Parameters*	*Day 1 (Baseline)*	*Day 3*	*Day 7*	*p value*
PZ1	TDS	1800 ± 100	1766.67 ± 57.73	1700 ± 100	0.422
	pH	3.15 ± 0.28	3.14 ± 0.08	2.84 ± 0.12	0.251
	Temperature	30 ± 1.00	38 ± 1.73	34.67 ± 2.51	0.001
	Titrable acidity	0.17 ± 0.01	0.18 ± 0.002	0.20 ± 0.002	0.001
	Specific gravity	1.07 ± 0.001	1.07 ± 0.00	1.07 ± 0.00	0.422
RZ2	TDS	1800 ± 0.00	1800 ± 100	1733.33 ± 57.73	0.422
	pH	3.13 ± 0.28	2.94 ± 0.08	2.86 ± 0.12	0.251
	Temperature	30 ± 1.00	6.67 ± 0.57	7 ± 1.00	0.001
	Titrable acidity	0.18 ± 0.01	0.14 ± 0.01	0.20 ± 0.00	0.001
	Specific gravity	1.04 ± 0.01	1.06 ± 0.03	1.06 ± 0.03	0.592
RZ3	TDS	1250 ± 132.28	1183.33 ± 104.08	1160 ± 36.05	0.550
	pH	2.99 ± 0.06	2.99 ± 0.07	2.91 ± 0.09	0.401
	Temperature	29.67 ± 0.57	7 ± 1.00	6.67 ± 0.57	0.001
	Titrable acidity	0.12 ± 0.01	0.15 ± 0.00	0.18 ± 0.02	0.002
	Specific gravity	1.06 ± 0.04	1.04 ± 0.01	1.05 ± 0.04	0.696
SBZ4	TDS	1863.33 ± 714.86	1783.33 ± 28.86	1766.67 ± 388.37	0.012
	pH	2.95 ± 0.05	2.80 ± 0.11	2.65 ± 0.30	0.231
	Temperature	30 ± 1.00	6 ± 1.00	5± 1.00	0.001
	Titrable acidity	0.18 ± 0.01	0.17 ± 0.02	0.27 ± 0.04	0.009
	Specific gravity	1.06 ± 0.03	1.04 ± 0.04	1.05 ± 0.02	0.821
SBZ5	TDS	1866.67 ± 115.47	1860.33 ± 305.50	1856.67 ± 115.47	0.308
	pH	2.91 ± 0.01	2.82 ± 0.26	2.79 ± 0.29	0.364
	Temperature	30 ± 1.00	7 ± 0.57	6 ± 0.57	0.001
	Titrable acidity	0.13 ± 0.10	0.18 ± 0.01	0.22 ± 0.04	0.296
	Specific gravity	1.07 ± 0.02	1.07 ± 0.05	1.12 ± 0.07	0.531
PZ6	TDS	1900 ± 100	1895±102	1783.33 ± 104.08	0.254
	Ph	2.97 ± 0.02	2.81 ± 0.10	3.10 ± 0.31	0.234
	Temperature	29.33 ± 1.52	7 ± 2.51	6± 2.00	0.001
	Titrable acidity	0.17 ± 0.01	0.16 ± 0.02	0.19 ± 0.02	0.381
	Specific gravity	1.07 ± 0.01	1.08 ± 0.02	1.09 ± 0.04	0.646

Specific Gravity

Specific gravity, an indicator of dissolved solids and sugar concentration, remained generally stable across most treatments throughout the seven-day storage period. Minor fluctuations observed in pasteurized and refrigerated samples suggest gradual sugar utilization by micro-organisms or limited evaporation under storage conditions. However, an anomalous spike in specific gravity was recorded in sample SBS2 on Day 3(2.00; p=0.01). This unusually high value likely represents a measurement or transcription error, as soymilk typically exhibits specific gravity.

Colony Morphology and Gram Staining of Bacterial isolates

The number of bacterial isolates found in the soymilk and zobo samples were 8 according to the information revealed by table 6, which included Proteus mirabilis, klebsiella pneumonia, *Staphlococcus aureus*, *Bacillus spp*, Salmonella typhirium, *Enterobacter aerogenes*, *Shigella flexneri*, *Eshelichia coli.* The form was circular and irregular and the surfaces of some isolates were glistening, smooth, shiny and rough. The colour of some of the isolates were creamy, yellowish, greyish and whitish. The margin of the isolates were entire, lobate and fimbriae, the elevations were convex and low convex and the opacity was opaque, translucence and moist. The bacterial isolates were of both a positive rod negative rods as well as one positive cocci.

Fungal Isolates and Their Characteristics

Table 8 provided fungal analysis which supported the microbial degradation seen in the samples of Soymilk and zobo Zobo s. Fungal species identified include *Aspergillus niger*, *Penicillium spp*.**,** and *Candida* ***spp.***, all of which are known to contribute to spoilage and affect the sensory properties of beverages. *Aspergillus*

niger: This fungus was identified as a major contaminant in the Zobo samples, known for producing aflatoxins, which are toxic and carcinogenic .*Penicillium spp.*: Identified in multiple samples, this fungus is often associated with moldy spoilage and may indicate compromised storage conditions. The presence of toxic fungi such as *Aspergillus niger* in the samples highlights the importance of both microbial management and the use of proper preservatives to mitigate the risk of mycotoxin contamination in beverages. Fungal growth was predominantly observed in unpasteurized beverages, indicating that pasteurization helps to reduce fungal proliferation.

Antioxidant Activities

Temporal Degradation Across Assays and Treatments

Table 11: showed antioxidant stability, quantified via DPPH**,** FRAP**,** and ABTS assays, serves as a direct proxy for the retention of health-related bioactive compounds in beverages.

DPPH Assay- Thermolabile Antioxidants Degrade Rapidly

All samples experienced statistically significant reductions ($p < 0.001$**)** in DPPH scavenging capacity by Day 7. For instance; PZ1 dropped from 91.39% to 58.14%, PZ6 dropped from 91.07% to 53.07%.This uniform decline across thermally treated samples reflects the high thermolability of polyphenols**,** flavonoids**,** and vitamin **C**, major constituents in traditional fruit- or herb-based non-alcoholic beverages (Prior *et al*., 2005). Interestingly, refrigerated samples (e.g.**,** RS3**,** RS6**)** exhibited relatively moderate declines, confirming literature assertions that cold preservation is superior in retaining antioxidant capacity, largely by inhibiting oxidative enzymes (Kaur and Kapoor, 2001).

FRAP and ABTS

Differential Sensitivity and Preservation Profiles. FRAP values also declined but to a more degree, and only significantly in thermally exposed samples (PZ6: $p = 0.001$). This suggests that while redox potential is affected by temperature, FRAP is less sensitive to moderate degradation. ABTS values, notably, remained statistically unchanged ($p > 0.2$**)** across most treatments and days. This robustness hints at the higher stability of ABTS-reactive antioxidants or its greater resistance to degradation under heating, aligning with prior comparative studies on antioxidant assay behavior.

Table 10. Mean Values of Analyzed Physiochemical Properties of Pasteurized, Refrigerated and Sodium Benzoate treated soymilk samples within 7 days.

Samples	*Parameters*	*Day 1 (Baseline)*	*Day 3*	*Day 7*	*p value*
SBS1	TDS	2056.67 ± 125.03	2050± 215.17	2036 ± 121.65	0.091
	pH	5.38 ± 0.33	4.67 ± 0.15	3.94 ± 0.09	0.001
	Temperature	29.67 ± 1.52	29 ± 1.00	24.67 ± 2.51	0.028
	Titrable acidity	0.12 ± 0.05	0.13 ± 0.06	0.13 ± 0.06	0.988
	Specific gravity	1.20 ± 0.26	1.17 ± 0.14	1.07 ± 0.04	0.657
SBS2	TDS	2040 ± 144.22	2038.67 ± 152.75	2056.67 ± 51.31	0.210
	pH	5.34 ± 0.29	4.66 ± 0.29	4.84 ± 0.58	0.197
	Temperature	28.67 ± 1.15	38.33 ± 0.57	35.33 ± 1.53	0.002
	Titrable acidity	0.17 ± 0.11	0.25 ± 0.22	0.25 ± 0.13	0.788
	Specific gravity	0.98 ± 0.12	2 ± 0.03	1.16 ± 0.09	0.001
RS3	TDS	2030 ± 60.82	2025 ± 8.66	2033.33 ± 57.73	0.126
	pH	5.63 ± 0.09	5.60 ± 0.14	4.60 ± 0.01	0.001
	Temperature	29 ± 1.00	6.67 ± 0.57	7 ± 1.00	0.001
	Titrable acidity	0.19 ± 0.27	0.21 ± 0.06	0.29 ± 0.02	0.746
	Specific gravity	1.33 ± 0.46	1.31 ± 0.25	1.09 ± 0.04	0.584
PS4	TDS	2006.67 ± 11.54	2003.33 ± 251.66	1953.33 ± 50.33	0.804
	pH	5.63 ± 0.11	5.63 ± 0.11	4.48 ± 0.05	0.001
	Temperature	29 ± 1.00	22.33 ± 2.51	28.33 ± 1.15	0.001
	Titrable acidity	0.17 ± 0.07	0.07 ± 0.01	0.09 ± 0.04	0.447
	Specific gravity	1.15 ± 0.08	1.12 ± 0.07	1.17 ± 0.14	0.850
PS5	TDS	2133.33 ± 75.05	2090 ± 85.44	2066.67 ± 57.73	0.101
	pH	5.46 ± 0.13	5.44 ± 0.08	4.27 ± 0.16	0.000
	Temperature	30 ± 2.00	37.33 ± 2.51	35.33 ± 2.51	0.000
	Titrable acidity	0.05 ± 0.02	0.08 ± 0.02	0.07 ± 0.02	0.149
	Specific gravity	1.13 ± 0.06	1.08 ± 0.03	1.10 ± 0.03	0.410
RS6	TDS	2121.67 ± 70.05	2093.33 ± 115.47	2080 ± 192.87	0.279
	pH	5.61 ± 0.14	5.60 ± 0.09	4.91 ± 0.08	0.001
	Temperature	29.33 ± 0.57	7.33 ± 1.52	8 ± 1.73	0.000
	Titrable acidity	0.10 ± 0.08	0.08 ± 0.01	0.07 ± 0.02	0.863

Key: PZ1 =pasteurized zobo sample 1

RZ2= refrigerated zobo sample 2
RZ3= refrigerated zobo sample 3
SBZ4= sodium benzoate treated zobo sample 4
SBZ5= sodium benzoate treated zobo sample 5
PZ6= pasteurized zobo sample 6
SBS1= sodium benzoate treated soymilk sample 1
SBS2= sodium benzoate treated soymilk sample
RS3= Refrigerated soymilk sample 3
PS4= pasteurized soymilk sample 4
PS5= pasteurized soymilk sample 5
RS6= refrigerated zobo sample 6

Table 11. Mean Antioxidant activity of zobo and soymilk samples pasteurized, refrigerated and treated with sodium benzoate on the 1st day (baseline), 3rd day and 7th day.

Samples	Parameters	Day 1 (Baseline)	Day 7	*p* value
PZ1	DPPH	91.39 ± 1.58	58.14 ± 1.98	0.001
	FRAP	64.70 ± 2.00	51.46 ± 1.51	0.001
	ABTS	72.13 ± 9.48	62.65 ± 0.65	0.159
RZ2	DPPH	89.43 ± 0.64	50.68 ± 0.32	0.001
	FRAP	66.87 ± 1.44	54.81 ± 3.86	0.007
	ABTS	65.32 ± 2.26	63.95 ± 1.42	0.424
RZ3	DPPH	91.00 ± 0.44	63.75 ± 1.40	0.001
	FRAP	67.64 ± 1.63	66.43 ± 2.27	0.499
	ABTS	63.85 ± 1.75	64.31 ± 1.22	0.729
SBZ4	DPPH	90.88 ± 0.72	64.14 ± 1.84	0.000
	FRAP	67.07 ± 1.62	66.07 ± 1.67	0.495
	ABTS	64.98 ± 1.34	64.51 ± 1.10	0.666
SBZ5	DPPH	89.96 ± 1.17	64.39 ± 1.82	0.001
	FRAP	65.78 ± 0.92	66.43 ± 1.36	0.531
	ABTS	63.76 ± 1.81	63.87 ± 0.94	0.926
PZ6	DPPH	91.07 ± 0.14	53.07 ± 2.44	0.001
	FRAP	66.04 ± 1.60	51.94 ± 2.11	0.001
	ABTS	64.30 ± 0.75	63.33 ± 1.17	0.295
SBS1	DPPH	93.73 ± 0.56	64.63 ± 1.36	0.001
	FRAP	66.46 ± 1.16	64.74 ± 1.86	0.245
	ABTS	65.38 ± 0.15	65.72 ± 1.49	0.729
SBS2	DPPH	93.07 ± 0.81	64.11 ± 0.77	0.001
	FRAP	67.78 ± 2.07	65.98 ± 1.27	0.271
	ABTS	65.26 ± 0.23	65.69 ± 1.02	0.910
RS3	DPPH	94.05 ± 0.25	65.97 ± 2.67	0.001
	FRAP	65.97 ± 1.60	66.53 ± 2.83	0.779
	ABTS	67.39 ± 1.87	65.50 ± 0.91	0.190
PS4	DPPH	94.89 ± 0.81	63.72 ± 1.16	0.001
	FRAP	66.26 ± 1.35	63.37 ± 1.02	0.042
	ABTS	67.11 ± 1.36	65.69 ± 1.03	0.225
PS5	DPPH	94.03 ± 0.58	66.95 ± 2.26	0.0060
	FRAP	65.71 ± 1.31	64.91 ± 0.85	0.427
	ABTS	67.53 ± 1.61	65.29 ± 0.52	0.084
RS6	DPPH	93.00 ± 0.74	66.99 ± 1.78	0.001
	FRAP	65.88 ± 0.76	66.31 ± 2.30	0.630

Key:PZ1 =pasteurized zobo sample 1
RZ2= refrigerated zobo sample 2
RZ3= refrigerated zobo sample 3
SBZ4= sodium benzoate treated zobo sample 4
SBZ5= sodium benzoate treated zobo sample 5
PZ6= pasteurized zobo sample 6
SBS1= sodium benzoate treated soymilk sample 1
SBS2= sodium benzoate treated soymilk sample
RS3= Refrigerated soymilk sample 3
PS4= pasteurized soymilk sample 4
PS5= pasteurized soymilk sample 5
RS6= refrigerated zobo sample 6

Sensory Evaluation of soymilk and zobo sample

Soymilk Samples

Table 12 revealed that soymilk sample was just able to retain its milky colour, smell, smoothness in an ambient temperature for 2 days. Refrigeration was able to extend its texture, colour and smell to 3 days. Table 13 revealed that the colour, odour and texture of the soymilk was able to be maintained for 3 days in an ambient temperature and was extended to 4 days in a refrigerated environment. Table 14 showed that sodium benzoate treatment was able to able to maintain the texture, colour and smell for two days in an ambient temperature and extended the shelf-life to 3 days in a refrigerator. Summarily, at ambient temperature, soymilk samples retained acceptable sensory qualities (colour, odour, texture) for only two days, after which signs of spoilage such as curdling, off-odours, and colour darkening were observed. Refrigeration extended sensory acceptability to three days, while pasteurization and sodium benzoate treatment further prolonged stability to four and five days, respectively.

Zobo Samples

Table 15 indicated that the colour, odour and texture of zobo samples were maintained for 2 days in an ambient temperature after refrigeration and 3 days . Table 16 showed that pasteurization of zobo was able to maintain its texture, colour and smell for 4 days in an ambient temperature and 5 days in a refrigerated environment. Table.17 showed that sodium benzoate treatment was able to extend the shelf-life of zobo for 3 days in an ambient temperature and 4 days in refrigerated condition.

Comparative Analysis and Shelf-life Implication

Pasteurization combined with refrigeration performed the best in extending the shelf- life of both soymilk and zobo samples with the longest shelf life extension recorded in zobo samples. Preservation with sodium benzoate combined with refrigeration also performed better in shelf life extension than refrigeration alone. This points to further studies on the reason behind the longer shelf life extension in zobo samples.

Table 12. Sensory Evaluation of Refrigerated Soymilk samples across 7 days in ambient and refrigeration temperatures.

Days	Ambient Temperature			Refrigerated		
	Colour	**Odour**	**Texture**	**Colour**	**Odour**	**Texture**
1	White	No smell	Smooth	White	No smell	Smooth
2	White	Present	Smooth	White	No smell	Smooth
3	Cream	Present	Rough	White	No smell	Smooth
4	Cream	Rotten	Curdled and grainy	Creamy white	Smelling	Curdled and grainy
5	Cream	Rotten	Curdled and grainy	Creamy white	Smelling	Curdled and grainy
6	Cream	Rotten	Curdled and grainy	Creamy white	Smelling	Curdled and grainy
7	Cream	Rotten	Curdled and grainy	Creamy white	Smelling	Curdled and grainy

Table 13. Sensory Evaluation of Pasteurized Soymilk Samples Across 7 Days**.**

days	Ambient Temperature			Pasteurized		
	colour	**Odour**	**Texture**	**Colour**	**Odour**	**Texture**
1	White	No smell	Smooth	White	No smell	Smooth
2	White	No smell	Smooth	White	No smell	Smooth
3	white	No smell	Smooth	White	No smell	Smooth
4	Creamy brown	Smelling	Curdled and grainy	White	No smell	Smooth
5	Creamy brown	Rotten	Curdled and grainy	Slightly Creamy white	No Smelling	Smooth
6	Creamy brown	Rotten	Curdled and grainy	Creamy white	Smelling	Smooth and coagulated
7	Creamy brown	Rotten	Curdled and grainy	Creamy white	Smelling	Smooth and coagulated

Table 14. *Sensory Evaluation of sodium benzoate Soymilk Samples Across 7 Days.*

days	**Ambient temperature**			**Sodium Benzoate treated**		
	Colour	**Odour**	**Texture**	**Colour**	**Odour**	**Texture**
1	White	No smell	Smooth	White	No smell	Smooth
2	White	No smell	Smooth	White	No smell	Smooth
3	white	No smell	Smooth	White	No smell	Smooth
4	Crumby creamy	Slightly smelling	Curdled and grainy	White	No smell	Smooth
5	Crumby Creamy	pungent	Curdled and grainy	Creamy white	Smelling	Curdled and grainy

6	Crumby Creamy	pungent	Curdled and grainy	Creamy white	Smelling	Curdled and grainy
7	Crumby Creamy	pungent	Curdled and grainy	Creamy white	Smelling	Curdled and grainy

Table 15. Sensory Evaluation of Refrigerated zobo samples across 7 days in ambient and refrigeration temperatures

Days	**Ambient Temperature**			**Refrigerated**		
	Colour	**Odour**	**Texture**	**Colour**	**Odour**	**Texture**
1	Red	No smell	Smooth	Red	No smell	Smooth
2	Red	No smell	Smooth	Red	No smell	Smooth
3	Red	No smell	Smooth	Red	No smell	Smooth
4	Creamy red	Present	Curdled and grainy	red	No smell	Smooth
5	Creamy red	Rotten	Curdled and grainy	Creamy red	Smelling	Curdled and grainy
6	Creamy red	Rotten	Curdled and grainy	Creamy red	Smelling	Curdled and grainy
7	Creamy red	Rotten	Curdled and grainy	Creamy red	Smelling	Curdled and grainy

Table 16. Sensory Evaluation Ambient and Pasteurized Zobo Samples Across 7 Days

Days	Ambient Temperature			Pasteurized		
	Colour	**Odour**	**Texture**	**Colour**	**Odour**	**Texture**
1	Red	No smell	Smooth	Red	No smell	Smooth
2	Red	No smell	Smooth	Red	No smell	Smooth
3	Red	No smell	Smooth	Red	No smell	Smooth
4	Reddish brown	slight smell	Slightly curdled and grainy	Red	No smell	Smooth
5	Creamy red	Smelling	Curdled and grainy	Red	No smell	Smooth
6	Creamy red	Smelling	Curdled and grainy	Creamy red	Smelling	Curdled and grainy
7	Creamy red	Smelling	Curdled and grainy	Creamy red	Smelling	Curdled and grainy

Table 17. Sensory Evaluation of Zobo samples in Ambient temperature and Sodium Benzoate across 7 days

Days	Ambient			Sodium Benzoate treated		
	Colour	**Odour**	**Texture**	**Colour**	**Odour**	**Texture**
1	Red	No smell	Smooth	Red	No smell	Smooth
2	Red	No smell	Smooth	Red	No smell	Smooth
3	Red	No smell	Smooth	Red	No smell	Smooth
4	Reddish brown	Slightly smelling	Slightly rough	Red	No smell	Smooth
5	Creamy red	Smelling	Curdled and grainy	Creamy red	Smelling	Curdled and grainy
6	Creamy red	Smelling	Curdled and grainy	Creamy red	Smelling	Curdled and grainy
7	Creamy red	Smelling	Curdled and grainy	Creamy red	Smelling	Curdled and grainy

IV. Discussion

The microbial load analysis of the zobo and soymilk samples for 7 days on the interval of 48hrs and 72hrs check produced 8 bacterial isolates which include *Proteus mirabilis* was identified as a Gram-negative rod, it exhibited catalase-positive, motility, and urease activity, often associated with spoilage in beverages [21]. *Klebsiella pneumoniae*, a common Gram-negative pathogen that fermented glucose and produced acid and gas, suggesting its role in fermentation or spoilage. *Staphylococcus aureus* is a Gram-positive coccus, identified for its ability to ferment glucose and produce acid, known for causing foodborne illnesses [22].

Bacillus *spp* is known for its ability to form spores, Bacillus species were prevalent in the samples, particularly in Soya Milk (S1), indicating that spore-forming bacteria might resist pasteurization if not exposed to high enough temperatures [23]. *Aspergillus niger* was identified as a major contaminant in the Zobo samples, known for producing aflatoxins, which are toxic and carcinogenic [24]. *Aspergillus niger* contamination may result in aflatoxin B1 presence above 2 µg/kg threshold posing significant safety concern [25]

Penicillium spp identified in multiple samples, this fungus is often associated with moldy spoilage and may indicate compromised storage conditions. Molecular identification from zobo and soymilk samples include Isolate LA (*Penicillium sp*.) with 88**%** similarity to *Penicillium sp.* UF1 Isolate S6 (*Fusarium sulawesiense*) with 99.79% similarity Isolate SH (*Aspergillus niger*) with 95**.**69**%** similarity Isolate S1 .Isolate LA (*Penicillium sp*.) with 88**%** similarity to *Penicillium sp*. UF1 Isolate S6 (*Fusarium sulawesiense*) with 99**.**79**%** similarity Isolate SH (*Aspergillus niger*) with 95**.**69**%** similarity Isolate S1 (Salmonella enterica subsp. enterica serovar Typhimurium) with 99**.**79**%** similarities

Therefore, appropriate preservation techniques are essential to minimize microbial contamination, especially fungal growth. subsp. enterica serovar Typhimurium) with 99.79% similarity. These results highlight the diverse range of microbial contaminants in indigenous non-alcoholic beverages, some of which are pathogenic, such as *Salmonella* and *Escherichia coli*. The presence of fungal species like *Penicillium spp.*, *Aspergillus spp.*, and *Fusarium spp.* suggests a high risk of spoilage and potential health hazards.

The finding from the effects of preservation methods on the microbial growth proved that pasteurization + refrigeration outperformed other methods (sodium sorbate treatment+refrigeration and refrigeration alone) in reducing microbial growth in soymilk and zobo samples. This was observed in pasteurized soymilk sample 1 (S1P) which showed that 3rd day microbial count recorded total bacterial count of 1.3×10^3CFU/mL ± 9.2×10^2 CFU/mL, total yeast count of 9.7×10^3CFU/mL ±2.3×10^2 CFU/mL, no growth of mold and enteric bacteria .There was resurgences of microbial growth on (the seventh) 7th day which agreed with the work of [26]. Presence of organisms like *Bacillus spp and Aspergillus flavus* give reasons for resurgences in microbial growth after pasteurization since their spores are capable of escaping heat associated with pasteurization. The effectiveness of pasteurization was replicated in pasteurized and refrigerated zobo samples (PZ1 and PZ6) where in PZ1 day 3 microbial analysis showed a significant decline from total microbial count recorded in the 1st. 3rd day analysis showed that total bacterial count was zero, total yeast count was zero, total mold count was zero and no enteric bacterial count was recorded. This deferred greatly from (the first day) 1st day result which showed that total bacterial count was 4.98×10^3CFUmL± 2.7×10^2CFUmL, total yeast count of 1.5×10^3CFUmL ± 1×10^2CFUmL, no growth of mold and enteric bacteria growth. This suggests that pasteurization effectively inactivates vegetative cells but may be less effective against heat-resistant spores, emphasizing the need for temperature optimization

Treatment of soymilk sample with sodium sorbate as emplified in (S5SB) on the 3rd day recorded total bacterial count of 3.0×10^4CFUmL± 2.10×10^2CFUmL, total yeast count of 3.3×10^3CFUmL± 1.2×10^2CFUmL, no mold count and total enteric bacterial count was 2.4×10^3CFUmL ±1.0×10^2. Which differed considerably with day 1 analysis. Look up table to compare refrigeration with other methods. This result agreed with the work of [27]. It was observed that refrigeration alone cannot inhibit the growth of microorganisms but only sedate them of which they start growing after gaining their normal temperature for metabolism and growth. This was shown in refrigerated zobo sample(Z3RF) where 3rd analysis showed a total bacterial count of 3.2×10^4CFUmL ±1.1×10^2CFUmL, total yeast count of 3×10^3CFUmL±1.0×10^2CFUmĹ, no growth of mold and total enteric bacterial count of 3.0×10^3CFUmL±1.2×10^2CFUmL. The above observation was in agreement with the work of [28]

The colour, taste, texture and odor showed that the pasteurized zobo sample was safe till day 6. The same trend was recorded in Pasteurized sample 6 (PZ6). This was also confirmed by stability in total dissolved solids of the zobo sample 1 and 6 [29]. Pasteurization+ refrigeration performed best of the three methods judging from the decline in *pH,* titratable acidity, specific gravity and total dissolved solids. Physicochemical analysis of zobo sample 1 (PZ1) showed stability of *pH* from the 1st - 3rd day (3.15-3.14), showing that no significant metabolic activity or fermentation went on. However it was recorded that on the 7th day of the analysis that *pH* declined to 2.48 showing that metabolic activities had set in. Physicochemical analysis of zobo sample 1 (PZ1) showed stability of *pH* from the 1st - 3rd day (3.15-3.14), showing that no significant metabolic activity or fermentation went on. However it was recorded that on the 7th day of the analysis that *pH* declined to 2.48 showing that metabolic activities had set in. Refrigerated zobo sample 2 showed a decline in *pH* from 3.13- 2.94 on the 1st and 3rd day respectively. Same trend was replicated in RZ3. The gradual decline in *pH* across refrigerated samples coincided with an increase in total yeast counts, indicating metabolic activity leading to organic acid accumulation—a hallmark of post-processing fermentation. Physicochemical analysis of zobo sample 1 (PZ1) showed stability of *pH* from the 1st - 3rd day (3.15-3.14), showing that no significant metabolic activity or fermentation went on. However it was recorded that on the 7th day of the analysis that *pH* declined to 2.48 showing that metabolic activities had set in. Sodium benzoate+refrigeration as a preservation method recorded a *pH* decline from 2.95-2.80 from 1st-3rd day showing an apparent metabolic activity resulting in production of organic acid. The 7th day analysis of SBZ4 showed a further decline in *pH* value . Pasteurized soymilk sample 4 (PS4) recorded no significant decline in *pH* value (5.46-5.45) from 1st to 3rd day and there was *pH* decrease on the 7th day indicating apparent metabolic activity. Same trend was recorded for pasteurized soymilk sample 5(PS5). This is in agreement with the work of Tomasula *et al.,*2011. Refrigeration soymilk sample 3(RS3) showed a significant decline of *pH* (5.63-5.50) from the 1st -3rd day respectively confirming the work of which showed that sodium benzoate + refrigeration did not measure up to pasteurization+refrigeration in preserving soymilk samples. Physical analysis showed that refrigerated soymilk samples maintained stability in taste, odour, colour and texture up to 4th day of the analysis. There was a further decline *pH* on the 7th day. Sodium benzoate+ refrigerated soymilk sample 2(SBS2) showed a decline in *pH* value (5.34-5.30) from 1st -4th day respectively. Further decrease in *pH* value happened in day 7. These are similar to the work of [30]

Findings proved that all samples experienced statistically significant reductions **($p < 0.001$)** in DPPH scavenging capacity by Day 7. For instance; PZ1 dropped from 91.39% to 58.14%, PZ6 dropped from 91.07% to

53.07%The sharp DPPH reduction in pasteurized samples suggests polyphenol oxidation induced by heat exposure, consistent with the thermal breakdown kinetics reported by Prior et al. (2005)." This uniform decline across thermally treated samples reflects the high thermolability of polyphenols**,** flavonoids**,** and vitamin C, major constituents in traditional fruit- or herb-based non-alcoholic beverages [31].Interestingly, refrigerated samples (e.g., RS3**,** RS6**)** exhibited relatively moderate declines, confirming literature assertions that cold preservation is superior in retaining antioxidant capacity, largely by inhibiting oxidative enzymes giving similar result with the work of [32] . FRAP values also declined but to a lesser degree, and only significantly in thermally exposed samples (PZ6: $p = 0.001$) statistical data for ANOVA. This suggests that while redox potential is affected by temperature, FRAP is less sensitive to moderate degradation. ABTS values, notably, remained statistically unchanged **(p > 0.2)** across most treatments and days. This robustness hints at the higher stability of ABTS-reactive antioxidants or its greater resistance to degradation under heating, aligning with prior comparative studies on antioxidant assay behavior affirming a similar claim by [33]

This work x-rayed that combined preservation methods like refrigeration+pasteurization performed the most in preserving locally made non-alcoholic beverages like soymilk and zobo hence best fit for longer shelf-life and edibility fitness. Among the evaluated preservation techniques, pasteurization combined with refrigeration demonstrated the most effective control of microbial proliferation and stability of physicochemical and antioxidant parameters. However, spore-forming and heat-resistant organisms indicate that process optimization or inclusion of natural antimicrobials could further enhance product safety and shelf life.

V. Conclusions

Pasteurization followed by refrigeration proved to be the most reliable way to preserve Zobo and Soymilk, effectively suppressing microbial growth, stabilizing physicochemical properties, and retaining sensory quality throughout storage. Refrigeration alone and sodium benzoate with refrigeration offered only moderate protection and allowed gradual quality decline. Heat treatment reduced some antioxidant measures (DPPH and FRAP) but had minimal impact on ABTS, reflecting varied thermal sensitivity among antioxidants. Overall, combining pasteurization with cold storage offers a practical, safe strategy for extending shelf life, with future work needed on integrating natural preservatives or controlled fermentation to further improve stability and nutrition.

Author Contribution

The research was conceptualized and supervised by Okafor U.C. The Data collection, analysis, and experiment were carried out by Ikebuaso S.I. The writing and arrangement of final manuscript were done by Ikebuaso S.I and Okafor U.C. Both authors read and approved the final manuscript.

Competing Interests

The authors hereby declare that competing interests do not exist regarding the publication of this research.

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