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# Effects of Preservatives and Storage Temperature on the Physicochemical, Microbial and Sensory Properties of Sharba-Milk (A Nigerian Indigenous Soft Drink)

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#### Abstract:

Storage potential of sodium benzoate (SB) and citric acid (CA) treated and pasteurized Sharbamilk stored for fifteen days under ambient and refrigerated storage were periodically studied. Proximate composition of the raw rice and Sharba milk (fresh)were respectively, moisture, 10.70% and 77.66%; protein, 6.49% and 3.92%; fat, 1.32% and 0.08%; ash, 0.49% and 0.04%; carbohydrate, 80.73% and 18.29% and food energy, (kcal/100g) 360.76 and 89.38. p Hand total soluble solids(TSS) decreased and titratable acidity (TTA) increased progressively during storage, more in the untreated control than in the treated samples. The levels of pH,TTA, total plate count(TPC),mould-yeast count (MYC), Coliform count, and TSS of Sharbamilk (control and treated)varied narrowly at the beginning and were: pH, 4.70-4.74; TTA, 0.31-0.72%; TSS, 16.41% - 16.65%; TPC, 1.02×10² - 8.20×10²cfu/ml; MYC, 1.60×10² - 2.31×10²cfu/ml; Coliform count, 1.02×10¹ -1.38×10¹.cfu/ml. TPC, MYC and Coliform counts of the untreated drinks under ambient storage climaxed before 10th day respectively: 3.34×10³,6.24×10³ and 2.52×10².A combination of SB and CA afforded the best protection from spoilage under refrigerated storage; neither TPC,MYC nor Coli form count exceeded10³cfu/ml in these drinks at the end of storage. Coli form activity was suppressed in all the drinks especially the drinks under chilled storage. The taste, flavor and acceptability of the drink sat the end of storage declined from their initial high values at the beginning. It could be concluded that storage life of sharba milk could be extended beyong15 days if treated with a combination of SB and CA coupled with chilled storage.

Keywords: Rice-based beverage, spices, chemical preservatives, pasteurization, chilled storage

#### 1. Introduction

Sharba-milk is a non-alcoholic beverage produced from rice grain (Oryza sativa), an indigenous drink produced and consumed by the Kanuri and Shuwa-Arab tribes of northeastern Nigeria. In its production, rice grains are soaked, wet-milled with desired spices (cloves, black pepper, ginger, chili pepper etc.), wet sieved, portioned into two, one part treated with hot water, mixed, cooled and combined with untreated portion, then sweetened and flavored with lime juice, chilled and served as a refreshing drink on festive occasions. The drink is produced and consumed on the same day because it is not shelf-stable, its sensory attributes deteriorate rapidly without refrigeration under tropical conditions. The use of known preservatives mainly organic acids or their salts to extend the storage life of Shaba – milk as in the case of commercial soft drinks has not been undertaken. It is known that fermenting activities of micro-organisms especially lactic acid bacteria leads to reduction in total solids, pHor increase intractable acidity although it hinders further microbial proliferation on a short-term basis (Banigoet al.,2015; Fasoyiro et al.,2005; Das, 2009), but it in-turn affects negatively both the nutritive, safety and sensory properties of the drink. Fungi especially yeast can still grow at reduced pH and chilled storage causing deteriorative changes in unpreserved soft drinks(Krgiel,2015). The recognition of the importance of probiotics which hitherto are obtainable from consumption of fermented dairy products has been stressed as a barrier to infective and non-infective disease conditions, the same benefits have been reportedly identified in cereal-based fermented drinks common in developing countries (Enujiugha and

Badejo,2007;Mokena Stall,2006). Citric acid (E 330) is the first choice for use in beverage preservation because of its high solubility, unlimited daily intake and flavor enhancing property apart from being an acid regulator and an anti-oxidant (Kregiel, 2015) yet it is less effective than other anti-microbials. Sodium benzoate (E211) on the other hand is a potent inhibitor of yeast than mould and bacteria, however citrates, sorbates, benzoates or their salts alone or in combination work well in acidic conditions (pH 2.2-4.4) enhancing the effectiveness of heat treatment since bacteriaceae in general are acid intolerant (Lawlor et al,2009). Formation of benzene a carcinogen from benzoate in drinks on extended storage at elevated temperature in presence of vitamin Chas raised concerns on its use (EPA, 2012), however wide scale use of this preservative is yet to be abated and is commonly available in Nigerian markets. Without the use of chemical preservatives many local soft drinks get fermented spontaneously leading to spoilage and unacceptability, therefore their production and consumption will remain localized at the household level. Therefore, the aim of this study is to evaluate the effect of two storage temperatures on the physiochemical, microbial and sensory properties of sharba milk treated with various concentrations of citric acid, sodium benzoate or their combinations.

#### 2. Materials and Methods

#### 2.1. Material Collection

The raw materials used for this study were obtained from Maiduguri Monday market, which included milled rice (dikwa long grain variety), chilli pepper, clove, black pepper, lemon juice, granulated white sugar). The chemicals / reagents used were of analytical grade and were sourced from Food Processing Laboratory of the Department of Food Science and Technology, University of Maiduguri, Nigeria. Fifty centiliter (cl)) polyethylene terephthalate (PET) bottles were sourced from the same market.

#### 2.2. Sample Preparation

The spices were individually cleaned by winnowing, later sorted to remove foreign matter. The spice mix contained the following spices: clove, black pepper, chili pepper, ginger in the ratio of 2:1:1:1, were blended together to become a powder. The rice grains were washed, then soaked(1:2 w/v) for five hours, aimed to soften the grains; washed rice grains were wet-milled in an attrition mill, the resulting slurry was wet-sieved with a muslin cloth to obtain a milky filtrate, which was portioned into two unequal portions(2:1); to the smaller portion warm water(80-85 'C)was added, twice its volume, mixed, left to cool for 40 min, later it was mixed with the larger portion, mixed and allowed to stand for 30 min. The entire filtrate was sweetened, flavored with lemon juice and pasteurized by warming at constant temperature range of 80-85°C for 30mins. The entire drink was portioned into seven hot-water cleaned containers to which right amounts of each preservative was added. Each portion was hot-filled (50cl) into individual hot-water sterilized PET bottles bearing labels corresponding to the concentration of the preservative used as shown here: A=control; B=0.1%SB; C=0.1%C; D=0.15%SB; E=0.15%CA; F=0.1%SB+0.15%CA.

One set of the sealed PET bottles were left at ambient temperature  $(T_a)$  (28±2°C), and the other set left under refrigerated storage  $(T_r,4-8$ °C), for 15 days. The flow diagram for the production of treated and control is given in Figure 1.

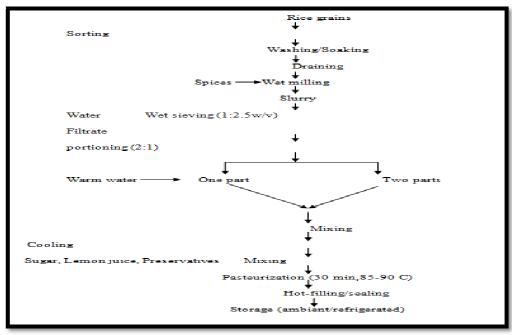


Figure 1: Flow Diagram Illustrating Sharba Milk Production

Ingredient	Quantity
Raw rice	1500g
Sugar (granulated)	150g
Ginger (powder)	10g
Cloves (powder)	20g
Black pepper (powder)	10g
Chili pepper (powder)	10g
Lemon juice	150ml
Water	4000ml
Sharba milk volume	3500ml

Table 1: Recipe for Sharbamilk Formulation

#### 2.3. Physiochemical Analysis

 $P^H$  was determined in clear extract of the drinks using a  $p^H$  meter ( $p_H$  – 920 LIDA instrument, shanghai, China) calibrated using phosphate buffer pH 4 and 7

The titrat able acidity (TTA) was determined according to the methods of AOAC (2000) using 20ml of the filtrates titrated against 0.1m NAOH using 2-3 drops of 1% phenolphthalein as the indicator, % TTA was calculated on the basis of lactic acid using the formula:

% TTA = litre value × 0.1MNaOH × 0.90 × 100/vol. of filtrate

Where 0.90 is the milli-equivalent of lactic acid.

The total dissolved solids (TSS) was determined using a handheld refractometer (REF-512, SCHMIDT-HAENSCH, GmbH). The refractometer was calibrated with a distilled water.

#### 2.4. Proximate Composition

The moisture, ash, protein  $(N\times6.25)$ , fat, millet seeds, rice seeds and of the freshly prepared sharba milk were determined by the methods of AOAC (2000).

Carbohydrates was determined by difference while the Food energy was calculated using At waters conversion factors for gross nutrients and expressed in kcal $\100$ g.

Moisture contents were determined by drying a weighed mass of the sample at  $105^{\circ}$ C for 2-3hours. Protein contents were determined using micro Kjeldahl method; the fat contents were determined by soxhlet extraction of a weighed sample using petroleum ether for 3h.

The ash contents were determined by incineration of weighed sample in a Muffle furnace at 550°C for 3h.

#### 2.5. Microbial Analysis

The microbial quality of the sharba milk during storage was determined according to the procedures of APHA (1984). The samples were serially diluted with 1% peptone water and 1ml was pour-plated on duplicate petri dishes each containing Nutrient agar for total plate count (TPC); Sabouroud dextrose agar (SDA) for mould/yeast count; McConkey agar for Coliform count. All plates were incubated at room temperature (28±2°C) for 3-5 days. Colonies were counted using a colony counter (Gallen Kamp, UK). Results were expressed in colony forming unit per milliliter (CFU/ml).

#### 3. Results and Discussion

Parameter	Rice grain	Sharba – milk
Moisture (%)	10.85±0.15	77.66±148
Protein (%)	6.49±0.21	3.29±0.10
Fat (%)	1.32±0.08	0.08±0.02
Ash (%)	0.6±0.12	0.05±0.01
Carbohydrate (%)	80.73±0.12	18.29±0.15
Food Energy (kcal)	360.76	89.38

Table 2: Proximate Composition of Milled Rice (Dikwa Variety) and Sharba Milk (Fresh)
Result Are Mean±SD of Triplicate Determinations

There were reductions in the mean proximate values of the nutrients in the Sharba-milk. Water content of the beverage was 77.66%, against 10.85% in the rice grain; protein, fat, ash and carbohydrate of the drink decreased remarkably from 6.49 to 3.92%, 1.32 to 0.08%, 0.61 to 0.05% and 80.73% to 18.29% respectively. Consequently, the energy value of the drink was very low (89.38kcal) against 360/76kcal in the grain. Hassan et al.(2012) reported the following values for rice flour: moisture content 12.33%, protein 8.3%, fat 1.60%, ash 1.38% and carbohydrate 87.1%. The same author obtained the following values for rice-based beverage before fermentation; protein 1.27%, ash 0.089%, fat 0.187%, carbohydrate 9.16%

and energy value 36.49 kcal/100g indicating even a higher decrease in nutrient content of the drink. Nkama et al.(1995) reported thatkunu-gyada (peanut supplemented cereal-based beverage) had: moisture 2.2-2.7%; fat 0.07 -1.0%; ash 0.08-0.09%; carbohydrate 10.60-16.0% and energy value 60-72 kcal.

This implies loss of nutrients in preparation of indigenous beverages from cereal grain, and if use is not found for the waste an environmental pollution would be likely for commercial scale production.

The Sharbamilk produced in this study had higher protein, carbohydrate and energy value than reported values. However, the fat and ash content were lower and this indicated that the drink would not develop rancidity during storage and supplementation with mineral elements would be needed.

Storage	Control	0.1%SB	0.1%CA	0.15%SB	0.15%CA	0.1%SB+CA	0.15%SB+CA
Day	Ta(Tr)						
0	4.70(4.72)	4.72(4.73)	4.73(4.73)	4.72(4.73)	4.72(4.73)	4.72(4.70)	4.72(4.74)
5	3.21(3.51)	3.60(3.69)	3.69(3.78)	3.60(3.68)	3.60(3.89)	4.00(4.14)	4.12 (4.21)
10	2.24(2.30)	2.44(2.62)	2.38(2.65)	3.39(3.51)	2.52(2.65)	2.84(2.93)	2.89(3.00)
15	2.00(2.04)	2.13(2.20)	2.09(2.13)	2.10(2.15)	2.20(2.23)	2.24(2.34)	2.30(2.40)

Table 3: Ph of the Control and Treated Sharba Milk Stored for Fifteen Days
Results Are Mean of Triplicate Determinations
SB = Sodium Benzoate, CA = Citric Acid, Ta = Ambient Temperature, Tr=Chilled Storage

There were general drop in the pH of the drinks, at day zero it varied from 4.70 to 4.74 for both control and treated samples, but at day fifteen the pH of the control at ambient and refrigerated conditions were the least 2.00 and 2.04 respectively; that of the treated samples at ambient and refrigerated conditions at day fifteen varied from 2.13 to 2.30 and 2.20 to 2.40 respectively. The samples with combined preservatives had greater pHof 2.24 to 2.40 than others at the end of storage. Activity of acid tolerant microorganisms would not be blamed alone for the very low levels of pH generally observed during storage, it could be attributed to lime juice addition during preparation of the drink in addition to the influence of added preservatives which are organic acids. Agarry et al. (2010) reported a pH range of 4.95 to 5.44 at zero hour which dropped to 3.78-3.94 six hours later; Danbaba et al. (2014) reported 6.31zero hour and 3.63twelve hours later for soybean-millet drink (kununzaki).

Storage	Control	0.1%SB	0.1%CA	0.15%SB	0.15%CA	0.1%SB+CA	0.15%SB+CA
Day	Ta(Tr)	Ta(Tr)	Ta(Tr)	Ta(Tr)	Ta(Tr)	Ta(Tr)	Ta(Tr)
0	0.7(NA)	0.60(NA)	0.31(NA)	0.37NA)	0.49( NA)	0.47(NA)	0.50NA)
5	1.33(1.09)	0.78(0.70)	1.04(0.75)	1.28(0.86)	1.06(0.86)	0.86(0.50)	0.70(0.49)
10	2.28(1.83)	1.48(1.26)	1.96(1.39)	2.18(1.92)	2.04 (1.93)	1.50(1.30)	1.38(1.39)
15	3.32(2.47)	2.49(1.57)	2.60(1.92)	2.40(1.96)	2.43(1.90)	2.52(1.96)	2.60(1.91)

Table 4: Titratable Acidity (%) Profile of the Control and the Treated Shaba-Milk during Fifteen Day Storage
Results Are Mean of Triplicate Determinations
SB = Sodium Benzoate, CA = Citric Acid, Ta = Ambient Temperature,
Tr=Refrigerated Temperature, NA=Not Available

Table4.0 indicates a general increase in the TTA of the stored drinks, more in the unprotected control and less in the protected refrigerated drinks. At day zero, the TTA of control at ambient was 0.72% which increased to 3.32% day 15, the highest TTA value; for the refrigerated control it was 2.47%. The TTA of treated drinks on ambient storage at day zero, varied from 0.31%-0.60%, lesser than in the drinks with combined preservative (0.1% or 0.15%SB+CA). At day 15, TTA values were still the least both for ambient and refrigerated storage. At day 5, TTA of the treated (ambient storage) ranged from 0.77% to 1.28% and for the treated (refrigerated) it was from 0.49% to 0.86%, but at day15 the TTA was 1.90-2.49% and 1.57-1.96% for ambient and refrigerated drinks respectively. Hassan et al. (2012) reported 0.08% for plain rice beverage at zero hour and 0.54% at the end of fermentation. The values reported in this study were higher probably because of the length of storage and the preservatives used and the implication was that the drinks were acidic, however, it aided to check the deteriorative activities of non- acid tolerant micro-organisms.

Storage	Control	0.1%SB	0.1%CA	0.2%SB	0.2%CA	0.1%SB+CA	0.2%SB+CA
Day	Ta(Tr)						
0	16.41(NA)	16.48(NA)	16.48(NA)	16.51(NA)	16.51(NA)	16.58(NA)	16.60(NA)
5	14.78 15.02)	15.32(15.68)	15.30(15.63)	15.58(15.69)	15.60(15.68)	15.63(15.78)	15.74(15.86)
10	11.18(11.32)	14.19(14.30)	14.30(14.17)	14.51(14.60)	14.50(14.05)	14.58(14.64)	14.76(14.85)
15	9.88(10.66)	11.28(11.41)	11.38(11.49)	12.77(13.01)	12.80(13.02)	12.42(12.53)	12.68(12.79)

Table 5: Soluble Solid (°brix) Profile of the Control and Treated Sharba Milk Stored for Fifteen Days
Results Are Mean of Triplicate Determinations
Key: SB = Sodium Benzoate, CA = Citric Acid, Ta = Ambient Storage,
Tr=Refrigerated Storage, NA=Not Available

There were reductions in total soluble solids(TSS) as shown in Table 5.0. The TSS of the drinks were in the of 16.41 to 16.65\*Brixday zero but at day 15 the TSS of control at ambient storage was 9.88\*Brixand that of the refrigerated,10.66\*Brix, the least reductions in TSS were observed in the refrigerated drinks with combined preservatives a narrow range of 12.53-12.79\*Brix, day 15 The soluble solids content of the treated refrigerated drinks varied from 15.32 to 15.86\*Brix day five; 14.19-14.85 \*Brix day 10 and 11.28-12.79 \*Brix day fifteen. Akomaet al. (2014) reported a range of 4.7 to 5.5 °Brix for commercial millet-based drink (kunun-zaki) sold in Bida metropolis, northcentral Nigeria, although a value of 9.07°Brix was obtained for the same drink (kununzaki) produced under laboratory conditions. Danbaba et al. (2014) reported TSS of 14.14°Brix at zero hour for millet-based drink (kununzaki) and the value dropped to 8.22°Brix after twelve hours. These reported findings are in tandem with the results obtained in this study and also indicates the protective action of the preservatives on the quality of the treated drinks. This is due to the fact that the treated samples had the least reduction in soluble solids during storage; the preservatives inhibit fermentative activity of micro-organisms that utilized soluble nutrients for growth, proliferation and production of other metabolites. Soluble solids represent the presence of soluble substrates such as sugars, amino acids, peptides etc.

	Paramet	Control	0.1%SB	0.1%CA	0.1%SB + CA
Storage Days		Ta(Tr)	Ta(Tr)	Ta(Tr)	Ta(Tr)
0	TPC	8.20x02 (NA)	1.10x10 <sup>2</sup> (NA)	1.26x10 <sup>3</sup> (NA)	1.02x10 <sup>2</sup> (NA)
	MYC	8.20x02 (NA)	2.10x10 <sup>2</sup> (NA)	1.86x10 <sup>2</sup> (NA)	1.60x10 <sup>2</sup> (NA)
	COL.	12 (NA)	12x10 <sup>2</sup> (NA)	14 (NA)	11 (NA)
5	TPC	3.14x10 <sup>5</sup> (5.30x10 <sup>3</sup> )	1.70x10 <sup>3</sup> (1.27x10 <sup>3</sup> )	1.48x10 <sup>3</sup> (1.2x10 <sup>3</sup> )	$1.06x10^{3}(1.02x10^{2})$
	MYC	4.1x10 <sup>5</sup> (2.30x10 <sup>3</sup> )	2.50x10 <sup>3</sup> (1.50x10 <sup>3</sup> )	$3.3x10^{3}(2.0x10^{3})$	1.16x10 <sup>3</sup> (1.11x10 <sup>2</sup> )
	COL.	1.39x10 <sup>2</sup> (7.8x10) <sup>1</sup>	4.6x10 <sup>2</sup> (2.2x10) <sup>1</sup>	$5.0 \times 10^{1} (2.4 \times 10^{1})$	18 (11)
10	TPC	3.34x108(4.1x104)	1.84x10 <sup>5</sup> (1.43x10 <sup>4</sup> )	1.84x10 <sup>5</sup> (1.43x10 <sup>4</sup> )	1.36x10 <sup>4</sup> (1.08x10 <sup>3</sup> )
	MYC	6.2x10 <sup>7</sup> (1.1x10 <sup>4</sup> )	4.3x10 <sup>4</sup> (1.10x10 <sup>3</sup> )	2.16x10 <sup>4</sup> (3.8x10 <sup>3</sup> )	8.56x10 <sup>4</sup> (1.08x10 <sup>3</sup> )
	COL.	2.52x10 <sup>2</sup> (74)	60(45)	45(18)	21 (18)
15	TPC	NA (6.24x10 <sup>4</sup> )	2.11x10 <sup>5</sup> (1.5x10 <sup>3</sup> )	2.9x10 <sup>4</sup> (15x10 <sup>3</sup> )	1.37x10 <sup>4</sup> (1.17x10 <sup>3</sup> )
	MYC	NA (4.16x10 <sup>4</sup> )	3.6x10 <sup>4</sup> (1.14x10 <sup>3</sup> )	2.24x10 <sup>5</sup> (2.17x10 <sup>3</sup> )	1.31x10 <sup>4</sup> (2.50x10 <sup>3</sup> )
	COL.	NA (83)	59 (32)	53 (21)	41 (18)

Table 6: Micro Flora Status of Preservative- Treated Sharba Milk Stored Under Ambient (Ta) and Refrigerated Temperatures (Tr)

Results Are Mean of Triplicate Determinations

TPC = Total Plate Count, MYC = Mould Yeast Count, COL = Coliform

SB = Sodium Benzoate, CA = Citric Acid, SB + CA = Combine SA and SB,

SB = Sodium Benzoate, CA = Citric Acid, Ta = Ambient

Temperature, Tr=Refrigerated Temperature

The general observation as seen in Table 6 was the activity of the microorganisms were not totally inhibited instead there were progressive increase in the population of the microbes in the course of storage especially in the untreated control /under ambient temperature storage, Coliform activity on the hand was suppressed but not in the untreated controls.

At day zero, the activity of the micro flora was barely notice able, the observed growth was caused by the time lag before analysis, and however it indicated an inclination for spoilage if the drinks were not placed under refrigerated storage. The TPC, MYC and Coliform counts of the untreated control before storage were  $8.20 \times 10^2$  cfu/ml,  $2.20 \times 10^2$  cfu/ml and 12 cfu/ml respectively; in the treated samples these values were even smaller and did not exceed regulatory limit (EC Regulation, 2073/2005). Thereafter, the activities of microflora heightened in all the drinks whether treated with preservatives or not, well pronounced in the drinks under room temperature storage.

The bacterial population of untreated control (ambient temperature=Ta) were  $3.14\times10^6$ cfu/ml day 5;  $3.34\times10^8$ cfu/ml day 10; that of MYC were  $4.1\times10^5$ cfu/mlday 5,  $6.2\times10^7$ cfu/ml day 10; Coliform  $1.39\times10^2$ cfu/ml day 5, and  $2.52\times10^2$ cfu/ml day 10. The drinks (control, Ta) had gone so bad before day 10, thereafter one of the bottled drinks exploded spilling its content indicating

gas build up as a result of yeast fermentation. Odu et al.(2012) reported that citric acid treated soymilk lasted up to 16 days while untreated control spoiled before 4 days. Adeola and Aworh (2014) reported sodium benzoate extended the shelf life of tamarind-based beverage under room and refrigerated storage by 6 and 13 weeks respectively and the untreated control deteriorated by second and tenth day room and refrigerated temperatures respectively. But the untreated control under refrigerated(chilled) storage(Tr) had minimal microbial activity, the bacterial population(cfu/ml) at day 5,10,15 were respectively  $5.30 \times 10^3$ ,  $4.10 \times 10^4$  and  $6.24 \times 10^4$ ; the MYC at day5,10,15 were  $2.3 \times 10^3$ ,  $1.1 \times 10^4$  and  $4.16 \times 10^4$ ; Coliform counts day5,10,15 were78,74 and 83.

The treated drinks under chilled storage had least microbial activity especially the drinks with combined preservatives (0.1%SB+CA or 0.15%SB+CA). The results obtained for 0.15%SB or 0.15%CA or a combination of them was excluded from the Table 6 for the purpose of avoiding a bulky table; however those values did not deviate strongly from values recorded for 0.1%SB or 0.1%CA or their combination.

For the drinks preserved with 0.1%SB, the highest TPC recorded throughout the storage period did not exceed  $10^5$  cfu/ml and  $10^3$  cfu/ml for ambient and chilled storage respectively; the highest MYC was recorded at day  $15^3$ . As and  $10^3$  Ta and Tr storage respectively; Coliform activity was insignificant at day 15.

Kregiel (2015) noted that spore-forming bacteria and yeast were responsible for spoilage of heat treated drinks under chilled storage. Coliform, non-sporulating gram negative bacteria could not withstand prevailing conditions during storage therefore their activity was inhibited. Presence of E, coli or Coliform indicates fecal contamination directly or indirectly; bacteriaceae are useful indicators of hygiene level, process adequacy and post processing contamination of heat processed foods(EC Regulation, 2073/2005). Decreased pH led to decreased population of the micro flora in the drinks but this effect is further strengthened by addition of chemical preservatives, this observation was confirmed by the high micro flora population of the control drinks left under ambient temperature in this study and those of workers: Okonkwo (2014) observed a drop in lactic acid bacteria population of millet-based drink from 11.5 ×10<sup>7</sup> pH 6.4 at zero hour to 5.75 ×10<sup>7</sup> pH 3.7 after 48 hours. Gotcheva et al.(2000) evaluated hawked boza, a Bulgarian cereal-based fermented drink and reported total plate count of  $8.6 \times 10^7 - 12.7 \times 10^7$ , LAB  $6.0 \times 10^7 - 8.8 \times 10^7$ , yeast/mold  $2.6 \times 10^7 - 3.9 \times 10^7$ , pH 3.2 - 3.5. Puerari et al.(2015) reported that chicha, a rice-based fermented beverage of the Brazilian's Amerindians had a bacteria population range of 01-6.83 logml-1, pH 5.2-3.9, between zero and 36 hours. Ramachandra and Nagarajan reported that 0.1% citric acid in addition to pasteurization and spice inclusion were responsible for storage stability of Aloe Gel - papaya fermented beverage. Lactic acid bacteria lose their viability under chilled storage more than most members of enterobacteriaceae, moreover members of this group are acid intolerant therefore lowered pH during storage of sharbamilk had inhibitory effect on their survival but the yeast was unaffected.

Drink type	Colour	Taste	Flavour	Acceptability
Control	8.07±0.98a	7.53±0.94 <sup>a</sup>	7.53±0.89a	7.83±0.53a
0.1%SB	7.67±0.88 <sup>b</sup>	7.13±0.86 <sup>b</sup>	7.33±1.24 <sup>ab</sup>	7.43±0.93 <sup>b</sup>
0.1%CA	7.67±0.96 <sup>b</sup>	7.33±0.92ab	7.30±0.88ab	7.47±1.40 <sup>b</sup>
0.15%SB	7.27±0.74 <sup>d</sup>	7.03±0.89ba	7.00±1.26ab	7.34±0.76bc
0.15%CA	7.43±0.94 <sup>c</sup>	7.27±0.83ab	7.23±0.82ab	7.37±0.85bc
0.15%SB+CA	7.23±0.82 <sup>d</sup>	7.37 <sup>b</sup> ±0.52 <sup>ab</sup>	6.87±1.17 <sup>b</sup>	6.97±0.89 <sup>c</sup>
0.15%SB+CA	7.53±0.86bc	7.33±1.09ab	6.93±1.01 <sup>b</sup>	7.47±0.86 <sup>b</sup>

Table 7: Sensory Scores of the Freshly Prepared Shaba-Milk (Control and Treated)
Results Are Mean±SD of Triplicates

Although significant differences (p<0.05) existed between the sensory attributes of the drinks (Table7) but in numerically terms the gaps were narrow, this was linked to flavor enhancing influence of the added preservatives only because the drinks were prepared from the same raw material, subjected to the same processing conditions. Citric acid and sodium benzoate as benzoic acid are known flavor enhancers or tartness inducers apart from their anti-microbial nature. (Krebs et al., 1983)

The untreated control maintained constant higher scores of the tested attributes indicative refusal by the panelist to abandon traditionally known sensory attributes of the Shaba milk; on the other hand, drinks with added combined preservatives had lower scores especially those with higher preservative concentration. The color of the fresh drinks was high (7-8) all tending towards a score of 8(very good). The taste and flavor of the drinks with higher concentration of combined preservative (0.15%SB+CA) were slightly inferior than in the untreated control. The overall acceptability of the drinks was high except drinks with 0.1%SB+CA for unknown reasons performed poorly.

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Drink type	Colour	Taste	Flavour	Acceptability
SB 0.1%	7.56±0.26a	4.21±0.10 <sup>d</sup>	4.21±0.09c	5.64±0.05 <sup>b</sup>
CA 0.1%	7.28±0.13a	4.15±0.08d	4.81±0.10 <sup>b</sup>	5.81±0.04b
SB 0.2%	7.64±0.08a	4.58±0.02c	5.17±0.03ab	6.18±0.06a
CA 0.2%	7.02±0.07ab	5.73±0.01 <sup>b</sup>	5.25±0.05ab	6.25±0.03a
SB+CA 0.1%	7.56±0.02a	5.13±0.10 <sup>c</sup>	5.25±0.04 <sup>ab</sup>	6.27±0.04a
SB+CA 0.2%	7.85±0.05a	6.63±0.23a	6.27±0.16a	5.92±0.11 <sup>a</sup>

Table 8: Sensory Scores of Treated and Refrigerated Shaba Milk Stored for 15 Days Results Are Mean±SD of Triplicate Determinations Key: SB = Sodium Benzoate, CA = Citric Acid

The sensory scores of treated and refrigerated Sharba milk kept for 15 days (Table 8) indicated that storage had no observable negative effect on the color(creamy milk) of the drinks but the taste and flavor scores declined in the course of storage, the taste scores varied 4-6, flavor from 4-7, it was observed that drinks with combined preservatives performed better in this case unlike in fresh drinks in Table 7.The acceptability scores declined more in the drinks treated with single preservatives than those with combined preservatives however they were not rejected although some had alcoholic flavor or taste, which was responsible for the lower scores.

#### 4. Conclusion

Dry matter content as well as food energy of sharba-milk were very low in relation to the values obtained for the main raw material, rice grain. Greatest inhibition was exerted on bacterial and coliform activity but mold/yeast count increased therefore were responsible for spoilage of the untreated control under ambient storage in less than five days, and were also responsible for reduction of sensory scores at the end of storage at which taste and aroma were not tolerable but alcoholic and acidic. pH and total dissolved solids decreased while titrat able acidity increased throughout the storage period. Sodium benzoate in conjunction with citric acid coupled with refrigerated storage afforded the greatest protection against the activity of microorganisms, but had no bactericidal effect. At the end of storage period all drinks under refrigerated storage treated or not had lower sensory scores than fresh sharbamilk, the untreated refrigerated control had the least scores. Therefore, it could be said that the combined effect of sodium benzoate and citric acid coupled with pasteurization, addition of spices and refrigerated storage were responsible for extending the shelf life of sharba-milk beyond fifteen days.

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