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COMPARATIVE ASSESSMENT ON MICROBIAL AND SENSORY QUALITY OF TIGERNUT MILK TREATED WITH TWO CHEMICAL PRESERVATIVES

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ABSTRACT

This study evaluated the microbial and sensory qualities of tiger nut milk produced from tiger nut seed. It was pasteurized, preserved with addition of chemical preservatives (0.08% sodium benzoate and 0.08% sodium metabisulphite) during seven days storage at refrigerated storage 4^0C and ambient storage of 28 ± 2^0C. The total viable count range from $1.0x10^2$ to $5.3x10^3$ cfu/ml, *Staphylococcus* count range from $1.6x10^2$ to $2.3x10^2$ cfu/ml. Fungi count range from $1.0x10^2$ to $2.0x10^2$ cfu/ml. The result shows that microbial count of tigernut milk treated with sodium benzoate was lower on the day one and seven than the other samples. Microbes isolated were *Bacillus* spp., *Staphylococcus* spp., *Lactobacillus* spp., *Leuconostoc* spp., while the fungi were *Saccharomyces* spp., *and Aspergillus* spp., Overall assessment showed that all the counts were within acceptable microbiological limit of$2.0 x10^5$cfu/ml for dairy milk food. The sensory evaluation of the samples showed that tiger nut milk preserve with sodium metabisulphite were more appealing in terms of taste and had the highest overall accept ability while there was no significant difference ($P<0.05$) in colour, flavour and texture in all the sample . The result of this study has shown that sodium benzoate reduces the microbial load in all attribute and hence preserved the drink better.

Keyword; *microbial, sensory, tigernut, milk, chemical preservative*

INTRODUCTION

The search for lesser known and underutilize crops, many of which are potentially valuable as human and animal foods has been intensified to maintain a balance between population growth and agriculture productivity, one of such crop is tigernut *(Cyperus esculentus)*(Tunde-Akintunde and Oke, 2011;Moore 2005).Tigernut *(Cyperus esculentus)* belongs to the family Cyperaceae and the order, Commelinalis . It is found worldwide in warm and temperate zones, occurring in Southern Europe and Africa. Tigernut *(Cyperus esculentus),* also known in Nigeria as "Ayaya" in Hausa, "Ofio" in Yoruba and "Akiausa" in Igbo has three varieties (black, yellow and brown) which are underutilized due to lack of information on their nutritional potentials (Ọmodẹ *et al., 1995).* It can be eaten raw, roasted, dried, baked or be made into milk (Oladele and Aina, 2007; Sa 'id *et al,* 2017).

Tigernut can be processed into varieties of milk product and can be used by special people having milk allergic such as galactosemia and lactose intolerance. The nuts are soaked in water, wet milled, sieved, sweetened and flavoured. Soaking is a major unit operation because it can be used to reduce soluble anti-nutrients such as tannins and polyphenols (Wailkm *et al.,* 2014). Tiger nut milk is naturally sweet, creamy, and offer a luxurious rich and nutty flavor. Unlike milk made from almond or other nuts which are typically high in inflammatory omega-6 fatty acid, tiger nut milk is instead rich in mono saturated fat as well as mineral and vitamin C and E. Tiger nut was reported as a very healthy as it help in preventing heart attack thrombosis and activated blood circulation. It also helps to prevent cancer of the colon due to the high content of soluble ~~of~~ glucose. Tiger nut was usually reported to have a positive effect on cholesterol level due to high content of vitamin E. They are thought to be beneficial to diabetes and those seeking to reduce cholesterol or loose of

weight. Tiger nut can be taken by diabetics patients for its content of sucrose and starch content of arginine which stimulates the production of insulin (Belewu and Belewu, 2007). Tiger nut was found to be a good substitute for some other (plant) milk sources. The nuts are valued for their highly nutritious starch content, dietary fibre, carbohydrate (Mono, di and polysaccharides) (Umeric and Enebeli, 1997). The nut was reported to be rich in sucrose (17.4 to 20.0%), fat (25.50%), and protein (8%) (Kordylas, 1990). The nut is also very rich in mineral content (Sodium, Calcium, Potassium, Magnesium, Zinc and traces of Copper (Omode*etat.*, 1995). Research studies show that 100g Tiger–nuts contain 386 kcal (1635 kJ), 7% proteins, 36% fats (oils), 31% starch, 21% glucose, and 26% fiber of which 14% is non–soluble and 12% soluble (Muhammad *et. al.*. 2011.). Tiger–nuts are regarded as a digestive tonic having a heating and drying effect in digestive system and alleviating flatulence. They also promote urine production. The nuts are said to be stimulant and tonic and also used in the treatment of indigestion, colic diarrhoea, dysentery and excessive thirst(Udeozor and Awonorin, 2014).The nut is found to be ideal for children, the elderly and for sports men and women(Marthinez,2003).Tigernut milk can be consumed immediately or stored in refrigerator or treated with preservatives. Sodium benzoate and Sodium metabisulphite are among the most commonly used anti-microbial for improving storage stability. Their wide usage is due to their broad spectrum activity against yeast, mould, and bacteria as well as their non-alteration of food flavor (Fleet, 2003).

Sodium benzoate is the most active against yeast and bacteria and least active against mould. Level of the use is usually range from 0.05 to 0.1% by weight and it causes no deleterious effect as it readily eliminated from the body (Lindsay, 1996)Sodium benzoate is the sodium salt of benzoic acid. It is used as an

antifungal preservative in pharmaceutical preparations and foods. It has a role as an antimicrobial food preservative, a drug allerge.

Sodium metabisulphites an inorganic compound of chemical. The substance is sometimes referred to as *disodium*metabisulfite.It is used as a preservative and antioxidant in food. The acceptable daily intake is up to 0.7 milligrams per kilogram of body weight. Sodium metabisulfite oxidizes in the liver to sulfate which is excreted in the urine.

There were many attempts to industrialize the locally prepared tigernut beverage but the inability to preserve the drink for a long time without spoilage has been a major drawback. This study was conducted to extract milk from tigenutby comparing the chemical preservative on microbial safety to increase the shelf life and consumer acceptability of tigernut milk beverage

Material

Source of the Material

Fresh tigernuts were purchased from local market in Ilaro market,Ogun state. The equipment and chemical used were obtained from Food Technology Department, The Federal Polytechnic,Ilaro.

Sample Preparation

Fresh tubers of tigernut were sorted, nut and seed which may affect the taste and quality of milk extract were removed, washed and rinsed with portable water and used to produce milk.

Production of Tiger-Nut Milk

The method described by Udeozor(Figure 1) was modified for the extraction of tigernut milk. One kilogram(1kg) of the fresh tiger nuts was manually sorted and cleaned to remove foreign particles

and unwanted materials. The nuts were soaked in portable water at ratio nut: water (1:3) overnight. The soaked nut was milled into slurry using fabricated attrition several times with addition of 3 litres of water. The slurry was pressed using muslin cloth to extract the milk. The extracted liquor was homogenized using a blender and rapidly cooled and stored in a white sterile 4 litres plastic container. The extract milk was pasteurized at 72^{o}C for15 min using steam jacketed pan (pasteurizer) bottled when hot rapidly cooled.

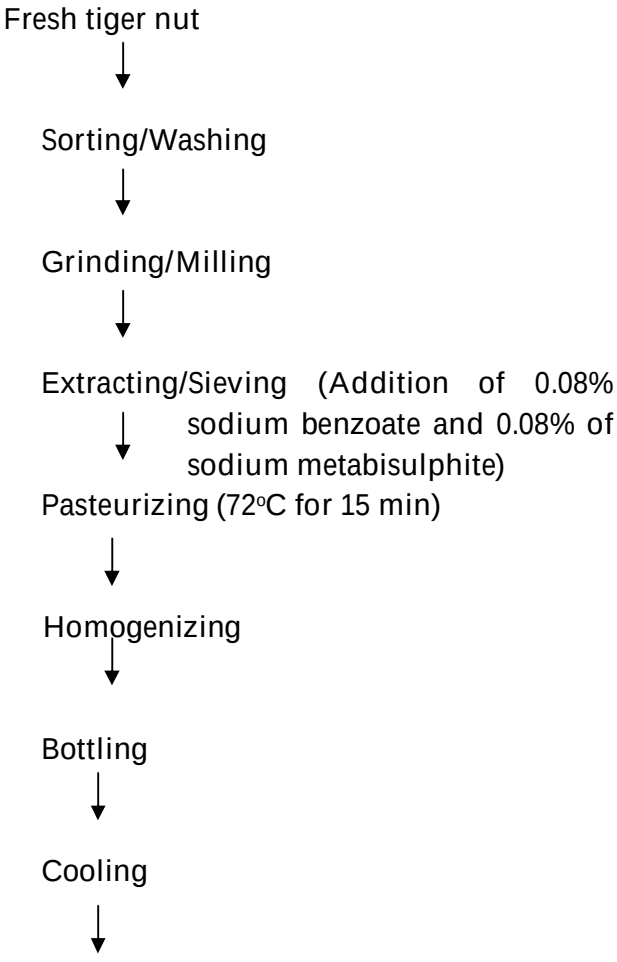

Fig 1. Flowchart of Tigernut Milk

Source: Udeozor and Awonorin(2014)

Microbial Analysis

One millilitre (1ml) of each sample was serially transferred into nine millilitres (9ml) of the sterile diluent (peptone water) with a sterile pipette and shaken vigorously. Serial dilution was continued until 10^6dilution was obtained.

Aliquot portion (0.1ml) of the 10^2 and 10^4 dilutions were inoculated onto freshly prepared, surface–dried nutrient agar (NA) and MacConkey agar (MCA) respectively. The same quantity (0.1ml) of the 10^2 dilution was inoculated onto potato dextrose agar (PDA). The inoculi were spread with a sterile (hockey stick–like) glass spreader to obtain even distribution of isolates after incubation. Nutrient agar and MacConkey agar plates were incubated for 24–48h at 37°C, *Staphylococcus* and *Salmonella* plates were incubated for 35°C while potato dextrose agar plate was incubated at ambient temperature (28± 02°C) for 3–5 days (Lynne,2003; Cheesbrough, 2002).

Sensory Evaluation

Ready to serve tigernut milk (a non-alcoholic beverage) was prepared in the processing laboratory of Food Technology Department. The tiger nut milk was presented to the panelists for sensory evaluation. The panelists evaluate the taste, texture, color, flavor and overall acceptability, using a Hedonic scale in accordance with method described by Larmond, (1977).

Result and Discussion

Result

Table 1: Microbial Analysis of Tigernut Milk Stored At Refrigerated Temperature

Sample	Total Plate Count	Coliform Countcfu/ml	Staphylococcus cfu/ml	Salmonella cfu/ml	Fungi Count cfu/ml
Day One	2.5×10^2	NG			
A			1.6×10^2	NG	1.5×10^2
B	1.0×10^2	NG	1.0×10^2	NG	1.0×10^2
C	2.0×10^2	NG	1.0×10^2	NG	1.0×10^2
Day Seven					
A	5.3×10^3	NG	2.3×10^2	NG	2.0×10^2
B	2.0×10^3	NG	1.5×10^2	NG	1.2×10^2
C	3.0×10^3	NG	1.7×10^2	NG	1.5×10^2

Table 2: Microbial Analysis Of Tigernut Stored At Ambient Temperature

Samples	Total viable count	Coliform count cfu/ml	Staphylococcuscfu/ml count cfu/ml	Salmonella cfu/ml	Fungi count cfu/ml
Day One	11.5×10^4				
A		NG	2.2×10^2	NG	4.0×10^2
B	5.1×10^4	NG	1.2×10^2	NG	2.3×10^2
C	7.8×10^4	NG	1.6×10^2	NG	3.5×10^2
Day Seven					
A	32.0×10^4	NG	3.7×10^2	NG	4.8×10^2
B	8.5×10^4	NG	1.7×10^2	NG	3.0×10^2
C	9.5×10^4	NG	2.5×10^2	NG	4.6×10^2

Key: Duplicate value of the analysis

Sample A- Tiger nut milk with non preservative

Sample B- Tiger nut milk with sodium benzoate

Sample C- Tiger nut milk with sodium metabisulphite

Table 3: Cultural and biochemical characteristics of bacteria isolated from storedTigernut milk

Suspected Microbes	Shape	Gram reaction	Catalase	Oxi dase	Coag ulase	Motility	Urease	Indole	Spore	Suc	Glu	Gal
Bacillus subtilis	Rod	+	+	+	-	+	+	-	+	+	+	+
Staphylococcusaureus	Cocci	+	+	-	+	-	+	+	+	+	+	+
Lactobaccilusplantarium	Rod	-	+	-	+	+	+	+	-	+	+	+
Pseudomonasflourescence	Rod	-	+	-	+	+	+	+	-	+	+	+

Table 4: The Sensory Evaluation of Tigernut milk

	Taste	Flavor	Color	Texture	Overall Acceptability
45OR	6.22^{b}	4.56^{a}	4.78^{a}	5.00^{a}	7.67^{a}
45CBEN	6.33^{ab}	5.00^{a}	5.44^{a}	5.44^{a}	6.56^{a}
45MET	7.69^{a}	5.67^{a}	5.56^{a}	5.89^{a}	7.89^{a}

~~**Table 4: The Sensory Evaluation of Tigernut milk**~~

Key:

45OR- Tiger nut milk with no preservatives

45CBN- Tiger nut milk with sodium benzoate

45MET- Tiger nut milk with sodium metabisulphite

DISCUSSION

Microbial Analysis

The microbial analysis of three different samples of tiger nut milk during storage is shown in Table 1.The total viable count of pasteurize and treated samples at day one of refrigerated sample range from 1.0 x10^{2} to 2.5 x 10^{2} cfu/ml, *Staphylococcus*1.0x10^{2} ^{2}to 1.6x10^{2} cfu/ml, fungi count range 1.0x10^{2} to 1.5x10 ^{2}cfu/ml with no growth of coliform and *Salmonella* both the refrigerated and ambient temperature.

At the 7th day, the total viable count range from 2.0x$10^{3}$to 5.3 x 10^{3} cfu/ml, *Staphylococcus* 1.5x 10^{2} to 2.3x10 ^{2}cfu/ml and fungi range from 1.2x10^{2} to 2.0x10^{2}cfu/ml. The tigernut milk stored at ambient temperature shows an increase in the count .The total viable count range of day one was 5.1x10^{4} to 11.5x10^{4} cful/mlwhile

on the 7th day, the total viable count was 8.5x10^4 to 32.0.x 10^{4}cfu/ml, *Staphylococcus* count was 1.2 x10^2 to 2.2 x10^2 and at seven day 1.7x10^2 to 3.7x10^2 cfu/ml and the fungi count range from 3.0x10 ^{2} to 4.8x10^{2}cfu/ml. The microbial analysis shows that tigernut milk treated with sodium benzoate was generally low in all the counts at both refrigerated and ambient temperature than the sample treated with sodium metabisulphite.

From the analysis all the count were recorded within the acceptable microbiological limit. ICMSF 1x10^6 cfu/ml for total plate count. However the *Staphylococcus*, and Fungal count increase subsequent days in contrast to the first day of preservation. The micro-organism isolated from tigernut milk treated with samples and control are presented in Table 3, it includes *Bacillus* spp., *Pseudomonas* spp., *Staphylococcus* spp., *lactobacillus* spp. and the fungi isolates were *Sacharomyces* spp. *and Aspergillus* spp. The presence of these bacteria in food samples in this study may be inevitable because the spores of some strains of these organisms are resistant to pasteurization temperature. *Bacillus* are spore-forming bacteria that are commonly found in soil, water (through soil-water contamination) and also on vegetables. *Pseudomonas sp*are able to grow on a wide variety of organic substrates and are regular components of food spoilage. The presence of *Staphylococcus* spp., in the milk could be attributed to its wide spread in the environment and could have been introduced after processing through cross-contamination(Dai *et al*., 2006). The growth of these microorganisms may be attributed to the cells of lactic acid bacteria that might have survived through processing treatments. In a study conducted Nyarko *et al*., 2011 on the assessment of microbiological safety of tiger nut in the cape c+oast metropolis of Ghana, it was observed that the most predominantly encountered species were *E. coli* and *Bacillus* spp. which had 18.9% each.

Sensory analysis; Table 4 shows the result of sensory evaluation of three sample of tiger nut milk pasteurized and treated with chemicals with the control stored under ambient and refrigerated temperature adjudged by a ten man panelist. The overall acceptability shown that sample C (treated with sodium metabisulphite)had the highest overall acceptability while sample B which was tiger nut milk treated with sodium benzoate has lowest overall acceptability.

CONCLUSION

Sodium metabisulpite and sodium benzoate have inhibitory effects on spoilage micro-organisms of tiger nut milk with sodium benzoate been the most inhibitory. The microbial status of samples after storage shows that preservatives added affected the quality of tiger nut milk differently. The microbial load was reduced compared to the tiger nut milk without preservative. Comparing the two preservatives showed that sodium benzoate preserved the tiger nut milk better than sodium metabisulphite in term of microbial spoilage whereas sodium metabisulphite added more appeal to the tiger nut milk concerning the sensory evaluation when compared to the nut milk preserved with sodium benzoate.

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