Effects Of Preservative Treatments On The Microbial And Sensory Composition Of Tigernut Milk

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Abstract- Tigernut milk drink is a nutritious milk-like aqueous extract produced from tigernut. Its availability is limited due to its short shelf life. The aim of this present study was to determine the effects of preservatives on the microbial and sensory quality of tigernut milk drink. Fresh tigernut milk drink was produced according to the standard procedure with sterile distilled water. The milk was divided into 5 portions, kept in a sterile container, preservatives were added according to the experimental design and kept at ambient and refrigerated temperature. The color, odor, taste and overall availability of the milk were observed daily for a period of five days. Fresh tigernut milk without any treatment spoiled within 24hours after production, while the portion supplemented with the different preservatives (benzoic acid, ginger extract, sodium metabisulphite and *Pleurotus ostreatus* extract), pasteurized and then stored under the different temperature had varying shelf life within the five days. The isolated microorganisms include; *Bacillus* sp, *Staphylococcu*s sp, *Lactobacillus* sp. This study revealed that tigernut milk can be preserved with chemical preservatives to extend its shelf life beyond 24hrs and up to 5days. Moreover, natural extracts can be used to improve the sensory properties.

Index term- Microbial, Preservatives, Sensory, Shelf life, Tigernut milk

I. INTRODUCTION

Increased awareness of the health and wellbeing of people globally have necessitated the partial switch from animal-based food products to natural and plant-based foods with nutrient balanced profile required for various metabolic, physiological and other functional demands (Panghal *et al*., 2018). Tigernut (*Cyperus esculentus*) is a plant of the division magnoliophyta, class liliopsida, order cyperales, family cyperaceae and genus cyperus (Ntukidem, 2019). It is known as a yellow nut sedge of the tropical and Mediterranean regions, it is not a real nut; despite its name but a tuber, however, its chemical composition shares characteristics with both tubers and nuts (Ukwuru and Ogbodo, 2011). It has three varieties (black, brown and yellow). The yellow variety is preferred because it yields more milk upon extraction, contains lower fat, less anti-nutritional factors especially polyphenols and more protein (Okafor *et al*., 2003 They are eaten raw or roasted, used as hog feed or pressed for its juice to make a beverage known as tigernut milk (Nwobosi *et al.,* 2013; Amponsaha *et al*., 2017).

Tigernut milk is a very nutritive and energetic drink, both for young and old, it is tremendously high in glucose, proteins and starch, it is also rich in minerals like Potassium, Phosphorous, Vitamins E and C. Tigernut milk has never been found to produce allergy but had been reported to be a healthy food, since its consumption can help prevent heart disease and thrombosis and is said to activate blood circulation (Chukwuma *et al.,* 2010; Ukwuru *et al.,* 2011; Udeozor and Awonorin 2014). It was also found to assist in reducing the risk of colon cancer (Elom and Ming, 2019). Tigernut milk is also a suitable drink for celiac patients, who are not able to tolerate gluten and also for the lactose-intolerant (Musa and Hamza, 2013; Gambo and Du'a, 2014). Tigernut milk has a very short shelf life which is less than 24 hours depending on the condition of storage (Akoma *et al.,* 2006). High temperature and humidity significantly reduce the shelf life of the product (Nutso, 2014). As a result, tigernut milk is often associated with significant microbial contamination, including bacteria and molds (Onovo and Ogaraku, 2007; Nutso, 2014).

Microbial contamination of tigernut-derived products usually occurs as a result of unhygienic processing, handling, transportation and exposure of these products (Ukpabi and Ukeye, 2015). Microbial contamination of tigernut-derived products could also be traced to microbial contamination of tigernut tubers used for production. Maduka and Ire, (2019) reported that the lack of effective antimicrobial treatments at any step from planting to consumption suggested that pathogens introduced at any point may be present on the final food production. The presence of these microorganisms in tigernut-milk is considered a threat to public health because they are capable of producing toxic metabolites which can cause ill health in humans.

Traditionally, food preservation has three goals; the preservation of appearance, the preservation of nutritional characteristics, and a prolongation of the time that the food can be stored. The preservative effect of ginger has been previously reported by Nwobosi et al., (2013). Ginger (*Zingiber officinale*) has antimicrobial and anti-mycotoxigenic effects, because of its aroma and taste it has been used for culinary purposes from ages. Ginger is also known to possess antioxidant properties.

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Oyster mushroom (*Pleurotus ostreatus*) is the most known functional food for human health (Oyetayo and Ariyo, 2013). Mushrooms are macro fungi that are edible with desirable culinary, nutritional, and medicinal characteristics, many mushrooms are poisonous or not palatable. *Pleurotus ostreatus* extracts and its isolated compounds can be used as antibacterial and antifungal agents, presumably they act as defense mechanisms against various microorganisms (Okafor *et al*., 2017).

Chemical preservatives reduce the microbial spoilage of foods by inhibiting the growth and proliferation of bacteria, yeasts and molds (Abdulmumeen *et al.,* 2012). Sodium benzoate (produce benzoic acid when dissolved in water) and benzoic acids are the most common used preservative. Sodium metabisulphite is an additive known to have fewer health hazards than other preservatives. Metabisulphites are reducing agents and may inhibit lipid oxidation. The antioxidant activity of sulphur dioxide is related mainly to the unbound non-ionic form. This study was carried out to assess the effects of chemical preservatives and natural extracts in the extension of the shelf life of tigernut milk.

II. MATERIALS AND METHODS

Sample Collection

Big yellow tigernut (the most commonly used for preparation of tiger nut milk) was obtained from Sasha market in Akure, Ondo State, Nigeria. The nuts were taken to the laboratory in a clean polythene bag for processing and analysis.

SAMPLE PREPARATION

Preparation of Tigernut drink

Tigernuts were sorted to remove dirt particles and spoilt nuts, washed with 40% alcohol to minimize contamination and prevent cell shrinking and then rinsed with sterile water. The nuts were then soaked for 12 h in sterile water at ambient temperature to soften the seed, blanched at 70°C for 5 min in order to inactivate enzymes that would likely cause clumping after extraction. One kilogram (1kg) of the nuts was then wet milled with 1000 mL of sterile water using a sterile laboratory blender and sieved with a muslin cloth. About 1000 mL of sterile water was added to the tigernut extract (1:1w/v, tiger nut versus water) was stabilized with 10 g of gelatin to prevent separation (Nwobosi *et al.,* 2013). The filtered extract was heated at 90 °C for 15 minutes, cooled to 4°C and refrigerated for further processes (Udeozor, 2012).

Preparation of ginger extracts

Fresh ginger was extracted after rinsing in 40% alcohol to reduce contamination and wet milled in a sterile blender (washed with 1% sodium hypochlorite) without water to obtain single strength extracts that were then filter-sterilized by millipore filter. The concentration level of ginger used in this study were based on allowable standard (citric acid, 540mg/L) as recommended by (Nwobosi *et al.,* 2013).

Preparation of mushroom extracts

The mushroom was washed under running water, oven dried at a temperature of 40°C. The dried samples were mashed and blended to form dry powder. The dry oyster mushroom powder was macerated with 95% ethanol with a ratio of 1:10. The liquid extraction obtained was filtered with Whatman No. 1 filter paper and then evaporated with a rotary evaporator.

Preparation of benzoic acid and sodium metabisulphite

In water 2.91 g/L of benzoic acid was dissolved at a mild temperature of 20 °C. The specific purity criteria of food additives levels are expressed as the free acid for use in nonalcoholic drinks is 150 mg/L. This correlates with (food and drug agency) FDA (2001) list of substances that are generally recognized as safe (GRAS) with a current maximum level of 0.1% in food. Sodium metabisulphite (5.56 g/L) was dissolved in water at a mild temperature of 20 °C. The specific purity criteria of food additives levels are expressed as the free acid for use in nonalcoholic drinks is 150 mg/L. This correlates with (Food and Drug Agency) FDA (2001) list of substances that are generally recognized as safe (GRAS) with a current maximum level of 0.1% in food.

MICROBIAL ANALYSIS

PLATE COUNT

The microbial contamination level was carried out in accordance with Cheesbrough (2006) using the plate count method. The milk samples were serially diluted (10^{-1} to 10^{-3}) after which 0.5 mL of the appropriate diluent was pour plated on each of the plates.

Bacterial enumeration was carried out on Nutrient agar (NA) for total bacterial count, MacConkey agar (MA) for total coliform count and the cultured plates were incubated at 37 °C for 24 hours. Fungal enumeration was carried out on Sabouraud dextrose agar for total fungal count and the cultured plated were incubated at 24ºC for 48 to 72 hours.

Isolation and identification of bacteria

Distinct isolates were selected from the plate counts media and were purified by sub-culturing aseptically on a nutrient agar media. The process was repeated thrice to purify the isolates. The isolates were Gram stained as described by Cheesbrough (2006). Biochemical tests (catalase, coagulase, oxidase, mannitol fermentation, motility and sugar test) were carried out to help in the identification of the isolates (Cheesbrough, 2006)

Isolation and identification of fungi

Isolates were selected randomly from the Sabouraud Dextrose Agar plates after taking counts and sub cultured aseptically on Sabouraud Dextrose Agar for 48 h. The process was repeated thrice to obtain pure isolates. An essential feature of fungal identification is the microscopic characteristics of the isolates using the Tease mount preparation. The mount was carefully examined under the microscope for the characteristic shapes and arrangements of the spores, hyphae, budding yeasts etc (Zafar *et al.*, 2017).

Sensory evaluation

Tigernut milk was stored for 5days. Tigernut milk samples with and without preservatives were subjected to sensory evaluation by a 20-member taste panel consisting of different groups of people who are familiar with beverages. Samples of tigernut milk was coded and presented to the panelist using white transparent disposable cups. Water was provided for mouth wash in-between evaluations. Panelists were asked to evaluate the samples for color, texture, taste and overall acceptability using a 9-point hedonic scale (9=like extremely and 1=dislike extremely) (Winschuket and O'Mahony, 2015). The sensory test on a hedonic scale was a means of elimination of the unwanted samples and the most preferred products. The sensory test was done on day 1 and 5.

Data analysis

Data gathered in the course of this study were subjected to Analysis of Variance (ANOVA) and standard error ± mean was used for comparison of means. Statistical significance was accepted at $p \leq 0.05$.

III. RESULTS AND DISCUSSION

The number of isolates obtained varies from one sample to the other, tigernut milk without preservative (TGM) had the highest bacterial count (59.0±0.01cfu/mL) while tigernut milk preserved with benzoic acid (TGB) had the least value of bacteria count (16.0±0.01cfu/mL) (Table 1). No coliform was observed except on day 5 from tigernut milk preserved with ginger extract (TGG) (Table 1). The number of microorganisms present in both ambient and refrigerated samples increased with duration of storage though refrigeration reduced the growth of bacteria and yeast up to five days. The highest bacteria load occurred in tigernut milk without preservative (TGM) and the lowest in tigernut milk preserved with benzoic acid (TGB). This indicated that the use of benzoic acid as a preservative agent reduced the growth of microorganisms in the tigernut milk sample compared to sodium metabisulphite, ginger extract and mushroom extract. This could be attributed to benzoic acid acting as an inhibitor of microorganisms by changing the internal pH of the microorganisms to an acidic state that is incompatible for the growth and survival of microorganisms (Kyzlink, 1998). Sodium metabisulphite was effective in prolonging the shelf life of tigernut milk. At ambient temperature tigernut milk maintained the growth and survival of microorganisms but there was a reduction in their occurrence when preserved with benzoic acid and sodium metabisulphite (Table 1).

Table 1: Microbial load of tiger nut milk, tigernut milk preserved with benzoic acid, sodium metabisulphite, ginger extract and mushroom extract (cfu/mL) in ambient and refrigerated storage condition

		DAY 1		**DAY 3**		**DAY 5**	
Samples		Amb	Ref	Amb	Ref	Amb	Ref
	TBC	18.0±0.01^{b}	6.0±0.01^{a}	35.0±0.01^{d}	18.0±0.01^{b}	59.0±0.01^{e}	22.0±0.01^{c}
TGM	TCC	NC	NC	NC	NC	NC	NC
	TYC	-	-	4.0±0.00^{b}	2.0±0.00^{a}	6.0±0.00^{c}	2.0±0.00^{a}

TGB	TBC	8.0±0.01[c]	3.0±0.01[a]	11.0±0.01[d]	7.0±0.01[b]	16.0±0.01[e]	7.0±0.01[b]
	TCC	NC	NC	NC	NC	NC	NC
	TYC	-	-	1.0±0.00[a]	0.0±0.00[a]	3.0±0.00[b]	1.0±0.00[a]
TGS	TBC	9.0±0.01[d]	5.0±0.01[a]	14.0±0.01[f]	8.0±0.01[c]	19.0±0.01[e]	7.0±0.01[b]
	TCC	NC	NC	NC	NC	NC	NC
	TYC	NC	NC	3.0±0.01[b]	1.0±0.01[a]	5.0±0.01[c]	3.0±0.00[b]
TGG	TBC	11.0±0.01[b]	6.0±0.01[a]	25.0±0.01[d]	16.0±0.01[c]	50.0±0.01[f]	29.0±0.01[e]
	TCC	NC	NC	NC	NC	2.0	NC
	TYC	-	-	5.0±0.01[b]	3.0±0.01[a]	5.0±0.01[b]	5.0±0.01[b]
TGM(po)	TBC	9.0±0.01[a]	12.0±0.01[b]	38.0±0.01[e]	18.0±0.01[c]	53.0±0.01[f]	37.0±0.01[d]
	TCC	NC	NC	NC	NC	NC	NC
	TYC	-	-	5.0±0.01[b]	4.0±0.01[a]	7.0±0.01[c]	5.0±0.01[b]

Values carrying the same alphabet in the same column are not significantly different (p>0.05).
Key: TGM-Tigernut milk; TGB-Tigernut milk with benzoic acid; TGS-Tigernut milk with sodium metabisulphite; TGG-Tigernut milk with ginger extract; TGM (po)-Tigernut milk with mushroom (*Pleurotus ostreatus)* NC; No count

IDENTIFICATION OF MICROBES

The following microorganisms were isolated from the tigernut milk viz: *Bacillus cereus*, *Staphylococcus aureus, Lactobacillus plantarum, Bacillus subtilis, Lactobacillus vini, Bacillus pumilus, Staphylococcus xylosus, Bacillus brevis* and *Lactobacillus composti.* Onovo and Ogaraku (2007) had earlier reported the presence of the above microorganisms in tigernut milk. There findings indicated that microorganisms are present in tigernut milk, microorganism like *Bacillus* sp and *Stapylococcus* sp may render the food unsafe for consumption. Tables 2- 6 indicate the microbial species isolated and identified from the tigernut milk samples.

Table 2: The Occurrence of the Bacterial Isolates in Tigernut milk Drink

Isolates	Day 1	Day 2	Day 3	Day 4	Day 5
S. aureus	+	-	-	-	-
B. subtilis	+	+	+	+	+
L. plantarum	-	+	+	+	-
B. cereus	-	+	+	+	+

Keys: *S. aureus – Staphylococcus aureus*; *B. subtilis- Bacillus subtilis; L. plantarum–Lactobacillus plantarum; B. cereus-Bacillus cereus;* + = positive;

Table 3: The Occurrence of the Bacterial Isolates in Tigernut milk preserved with Benzoic Acid

Isolates	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
S. aureus	+	+	+	+	+
L. plantarum	-	+	+	+	+

Keys: *S. aureus – Staphylococcus aureus*; *L. plantarum –Lactobacillus plantarum;* + = positive; - = negative

Table 4: The Occurrence of the Bacterial Isolates in Tigernut milk with Preservative Sodium metabisulphite

Isolates	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
S. aureus	+	-	-	-	-
B. subtilis	+	-	-	-	-
L. vini	-	+	+	+	+
B. cereus	-	+	+	+	+
B. brevis	-	+	+	+	+

Keys: *S. aureus – Staphylococcus aureus*; *B. subtilis- Bacillus subtilis; B. cereus-Bacillus cereus; L. vini –Lactobacillus vini; B. brevis-Bacillus brevis;* + = positive;- = negative

Table 5: The Occurrence of the Bacterial Isolates in Tigernut milk preserved with Ginger

Isolates	Day 1	Day 2	Day 3	Day 4	Day 5
B. subtilis	+	-	+	+	-
S. xylosus	+	-	+	+	+
L. vini	+	-	+	+	+
B. pumilus	-	+	-	-	-
B. brevis	-	+	+	+	+

Keys: *S. xylosus – Staphylococcus xylosus*; *B. subtilis- Bacillus subtilis; L. vini –Lactobacillus vini;B. brevis-Bacillus brevis; B. pumilus-Bacillus pumilus* + = positive; - = negative

Table 6: The Occurrence of the Bacterial Isolates in Tigernut milk preserved with *Plerotus ostreatus* extract

Isolates	Day 1	Day 2	Day 3	Day 4	Day 5
L. composti	+	-	-	-	-
B. subtilis	+	+	+	-	-
L. plantarum	-	+	-	+	+
B. cereus	-	+	-	-	-
B. pumilus	-	-	+	+	+

Keys: *L. composti- Lactobacillus composti; B. subtilis- Bacillus subtilis; L.plantarum –Lactobacillus plantarum; B.cereus-Bacillus subtilis; B.pumilus-Bacillus pumilus* + = positive; - = negative

SENSORY EVALUATION

The appearance of any product usually determines the level of its visual appeal and frequency of purchase and at such, colour is a vital determinant of food appeal and acceptability (Ma *et al*., 2015). Additionally, flavour, taste and texture also play an important role in food acceptability and quality attributes (Ikujenlola *et al*., 2019), therefore evaluating the sensory characteristics of the tigernut milk preserved with both chemical and natural preservatives helped determine the quality. All samples were acceptable for its colour, texture and taste (Fig 1), however tigernut milk preserved with ginger extract (TGG) and tigernut milk without preservative (TGM) were preferred to the other tigernut milk samples. This may be because of the appealing nature of the aroma and flavour of the ginger extract and also the familiarity in the taste of tigernut milk. FIG 5 indicates the decrease in the overall acceptability of the tigernut milk samples because of the actions of microorganisms leading to foul odour, separation of liquid from solid and lumpiness of the milk. Tigernut milk preserved with benzoic acid (TGB) and tigernut milk preserved with sodium metabisulphite (TGS) maintained the sensory appeal in terms of colour, taste and texture.

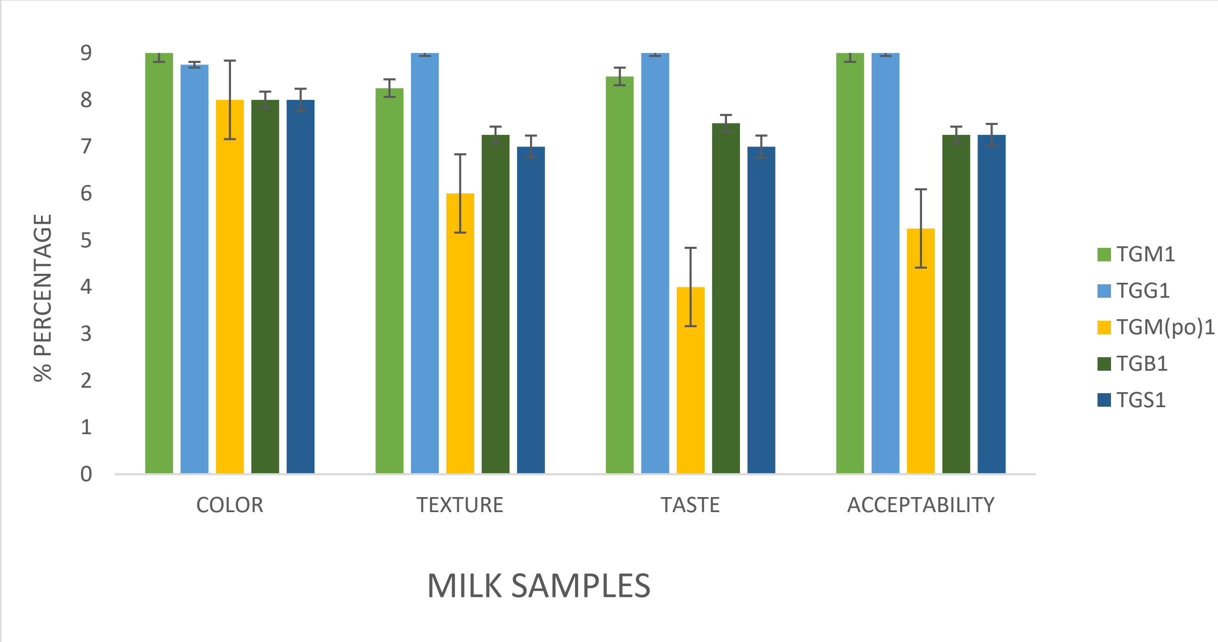

Figure1: sensory evaluation of the tigernut milk samples on day 1

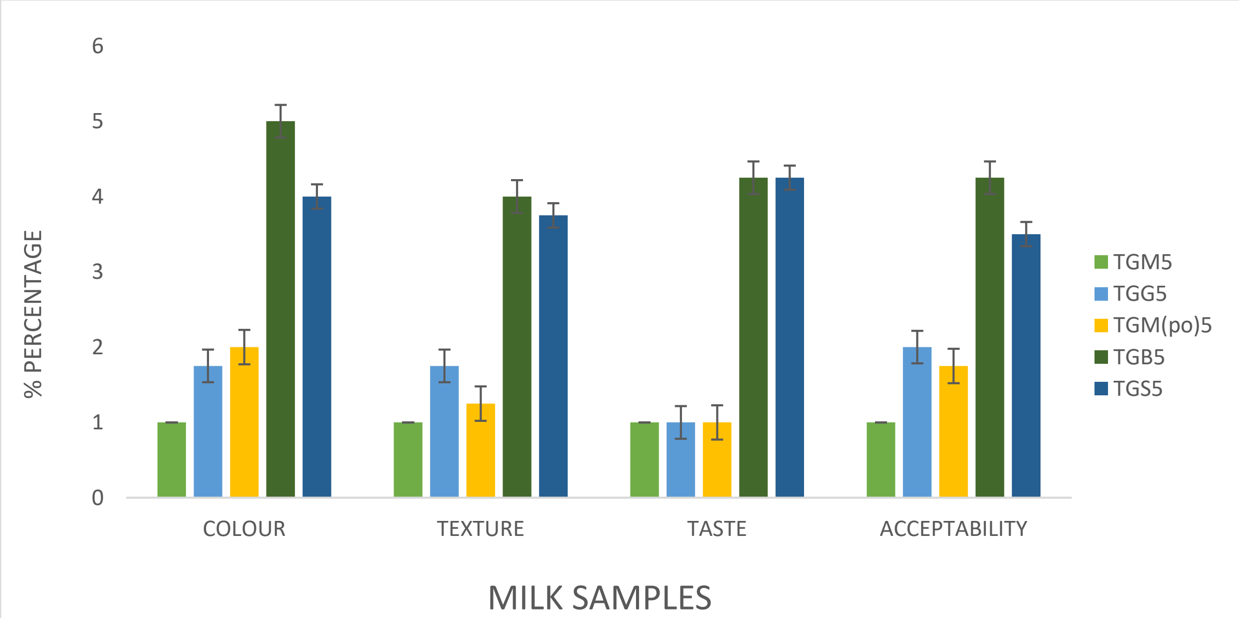

Figure 2: sensory evaluation of the tigernut milk samples on day 5

IV. CONCLUSION

The formulation of tigernut milk and all the processes involved were sufficient to produce tigernut milk that meet standard requirement. This study has revealed that tigernut milk preserved with benzoic acid and sodium metabisulphite has better general acceptability after storage and could be made available for public consumption. This study also shows a promising marketability potential of the non-diary beverages for vegetarians and individuals with lactose intolerance.

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