COMPARATIVE ANALYSIS BETWEEN PRESERVED AND UNPRESERVED TIGER NUT JUICE USING TWO DIFFERENT SPECIES (YELLOW AND BROWN)

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ABSTRACT: Studies on the preservation and proximate composition of tiger nut milk using two difference samples were carried out (Brown and Yellow) The aqueous extract of tiger nut was treated with ginger, pasteurized (800c for 5mins) and refrigerated (40c temperature) for 5 days, following which it was evaluated for its microbiological PH, proximate, mineral and sensory overall acceptability. The results obtained showed that there was a loss in nutrient content of the unpreserved tiger nut milk and subsequent increase in microbial count in the unpreserved Tiger nut milk, inadequate sterilization of utensils used also contributed to the presence of microbes.

INTRODUCTION

Tiger nut (Cyperus esculantus) is an annual or perennial crop belonging to the sedge family, native to warm-temperate to sub tropical regions, it can be found wild as weed or as a crop. It is cultivated as edible sugars called earth almonds or Tiger nut for the preparation of horchata de cufas; A sweet milk like beverage, It grows up to 90cm with solitary stems growing from a tuber. The plant is produced by seedling, creeping rhizomes and tubers. The tubers are between 0.3-1.9cm in diameter and the colors vary between yellow, brown and black. One plant can produce several hundred to several thousand tubers during a single growing season. Cyperus esculentus is wind pollinated and requires cross-pollination as it is self incompatible.

Tiger nut milk also called horchata de cufas is a refreshing vegetable drink and/or desert which is prepared with water, sugar and tiger nuts. It is very nutritive and energetic both for young and old, it is tremendously high in starch, natural fat, glucose and proteins. Also rich in minerals like potassium, phosphorous, vitamin E and C.

Tiger nut milk contains a large amount of oleic acid and cardiac preventive. It defends the intestinal mechanism and prevents both constipation and diarrhea.

Incase of soya milk or other soya products, tiger nut milk has never been found to produce an allergy. It contains no sodium, which makes it perfect for people with high tension.

Preserved tiger nut milk is the one submitted to a treatment of pasteurization without adding additives to ensure destruction of micro organisms , its composition is the same as the natural tiger nut juice. The aqueous extract of the tiger nut is to be treated with two natural tropical

preservatives (ginger) pasteurized for about 80o for 5 minutes and stored (ambient 28oC, refrigerated -4oC) for 5 days constant refrigeration. The micro biological, pH, proximate, mineral and sensory overall acceptability will be evaluated

While for unpreserved tiger nut milk is the one which is not subjected to treatment of pasteurization, it is the one prepared with the right amount of tiger nut. Water and sugar for the product to have minimum of 12% soluble solids, 2.2% of starch, 2.5% fats,6% pH and less of 10% of sugar in form of sucrose.

MATERIALS

Tiger nut (2 cups for each sample) Water Bucket (1bowl)Sieve (cloth)Blender Cooking pot, Stove, Stirrer, Ginger (2 slides), Sugar (to cooking spoons), crystal viclet, lugol lodine, Acetone, Distilled water, Neutral red, wire loop, spirit lamp

Sample and preservatives collection

Fresh tiger nut (Cyperus esculantus) of 2 different species (brown and yellow), fresh ginger (Zingiber officionale), dates was bought from local farmers in Maiduguri Monday market Borno State. These were kept at room temperature pending their use.

Preparation of tiger nut extract

Tiger nut were sorted to remove dirt's particles and spoilt nuts washed with water to minimize contamination and prevent cell shrinking. The nuts were then soaked for 48hours in water at ambient temperature to soften the seed and blanched at 70oc for 10min in order to inactivate enzymes that would likely cause clumping after extraction. Tiger nuts together with the mixture of date, coconut and ginger were then wet milled with clean water using a sterile laboratory blender pressed and sieved using cloth (0.01 diameter) with about 100ml of water following which the tiger nut extract (1.2w/v tiger nut versus water). Sugar and flavor are then added to taste. It is served chilled or packaged.

METHOD

Sample Processing

Serial dilution: serial dilution of the sample is carried out by using 9ml of normal saline into 8 sterile test tubes, 1ml of each of the tiger out juice sample (yellow & brown) was introduced into the test tubes containing 9ml of normal saline.

Preparation of Nutrient Agar:

28gl of nutrient agar were weighed with digital analytical balance, about 1liter of distilled water was boiled and the weighed agar was dissolved in it with constant stirring with the aid of glass rod to avoid the formation of lamps. The dissolved medium was poured into 1000ml (1liter) conical flask, and the volume was topped to the mark of 1 liter with the aid of distilled the 1liter mark. The flask orifice was coked with cotton wool and wrapped with aluminum foil, loaded into the autoclave and sterilize at the temperature than of 121^{o}C 15psi (pound per square inch) for 15mins. The medium was allowed to cool to about 45^{o}C before opening the autoclave to remove the medium.

Preparation of MacConkey Agar:

52gl of macconkey agar were weighed with digital analytical balance, about 1liter of

distilled water was boiled and the weighed agar was dissolved in it with constant stirring with the aid of glass rod to avoid the formation of lamps. The dissolved medium was poured into 1000ml (1liter) conical flask, and the volume was topped to the mark of 1 liter with the aid of distilled the 1liter mark. The flask orifice was coked with cotton wool and wrapped with aluminium foil, leaded into the autoclave and sterilized at the temperature of 121^{o}C, 15psi (pound per square inch) for 15mins. The medium was allowed to cool to about 45^{o}C before opening the autoclave to remove the medium.

Pouring of the Media into Sterile Petri dish:

The prepared medium was ascetically poured into sterile petri dishes by first sterilizing the on face of the conical flask over a spirit flame and about 10ml of the medium was poured into each plates, and allowed to solidify on the bench.Aseptic technique most be observed to avoid contamination from the environment, this is achieved by using 70% of ethanol mopped with a piece of cotton wool and the surfaces of the work bench, an also the oriface of the conical flask containing before flaming the oriface over the spirit lamp flame.

PH Determination:

PH samples was measured using a PH meter model radiometer A/S, Copenhagen, Den mark after standardization with buffer PH, 5,7 and 9 respectively.

Mineral Content Determination:

The mineral content of the product, calcium, Iron, Sodium and potassium, were evaluated using atomic absorption spectrophometer (AAS_ (perkins – Elmer model).

Proximate Analysis:

Total solids and moisture content of Tiger nut, was determined by the official air-oven method. The loss in weight was reported in percentage moisture. The dry matter or total solids was the weight obtained after drying the sample.

Nitrogen Determination:

The official micro-kjeldahl method was used to determine the nitrogen content of the tiger nut drink. Crude protein was calculated by multiplying the %Nitrogen by a factor of 6.25.

Crude fat Determination:

The fat content of the sample was determined according to the soxhlet extraction method. The round bottomed flask before and after extraction was recorded as the weight of the fat extracted.

Total ASH Content Determination:

The weight of the sample after igniting in the furnace was recorded as the ash.

RESULT

Inoculation of the serially diluted tiger nut juice samples,

About 1ml of the serially diluted tiger nut juice sample were aseptically pipetted with a pasture pipette and inoculated into a plate of nutrient agar (NA) and macConkey agar (MCCA) for each of the sample respectively

Incubation of the inoculated samples

These sample were incubated at ambient temperature for 24 hours. Then we cannot the colonies emerged from the incubated sample using colony counter as follow

Table-1. Total mold count at 24hour Incubation

S/N	Sample	Treatment/ serial dilution	CFU/1ml at 10^{-3} dilution
1	Yellow unpreserved	P D A 10^{-3}	6.42×10^{3}
2	Yellow preserved	P D A 10^{-3}	1.2×10^{5}
3	Brown preserved	P D A 10^{-3}	6.9×10^{5}
4	Brwn unpreserved	P D A 10^{-3}	2.3×10^{6}

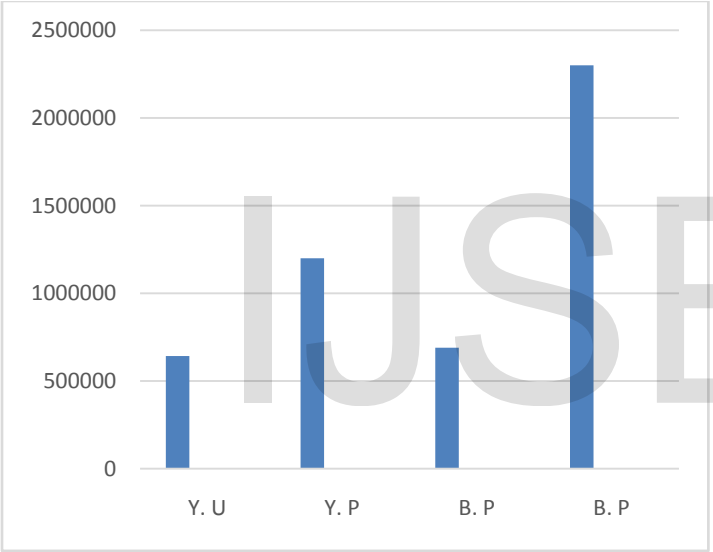

Figure-1.Total mould count at 24hours incubation

Table-2.Total mould count after 48 hours incubation

S/N	Sample	Treatment/ serial dilution	CFU/1ml at 10^{-3} dilution
1	Yellow unpreserved	P D A 10^{-3}	1.3444×10^{6}
2	Yellow preserved	P D A 10^{-3}	2.40×10^{6}
3	Brown preserved	P D A 10^{-3}	1.64×10^{6}
4	Brwn unpreserved	P D A 10^{-3}	1.42×10^{6}

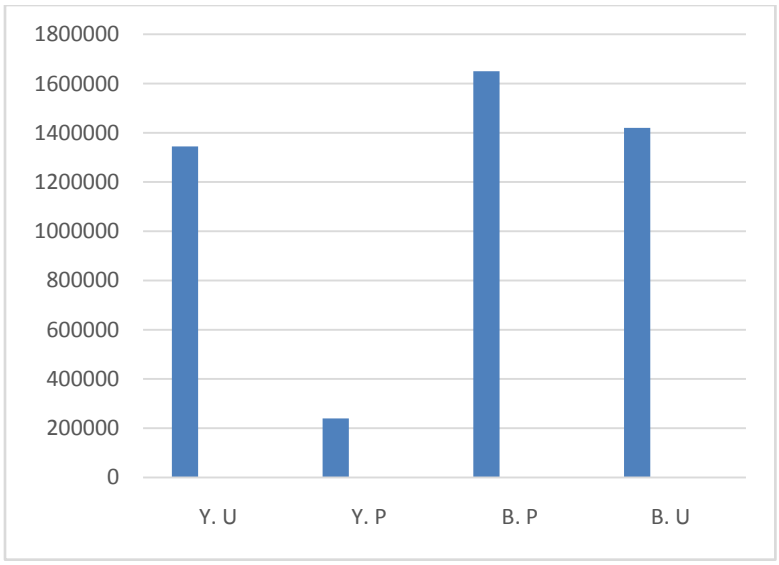

Figure-2.Total mould count at 48hours incubation

Table-3.Total viable bacterial count after 24 hour incubation

S/N	Sample	Treatment	CFU/1ml/serial dilution	Remark
1	Yellow unpreserved	NA 10^{-3}	248 x 10^{3}	White colonies
2	Yellow preserved	NA 10^{-3}	384 x 10^{3}	Yellowish white colonies
3	Brown preserved	NA 10^{-3}	340 x 10^{3}	Whitish colonies
4	Brwn unpreserved	NA 10^{-3}	4.3 x 10^{5}	Yellowish colonies

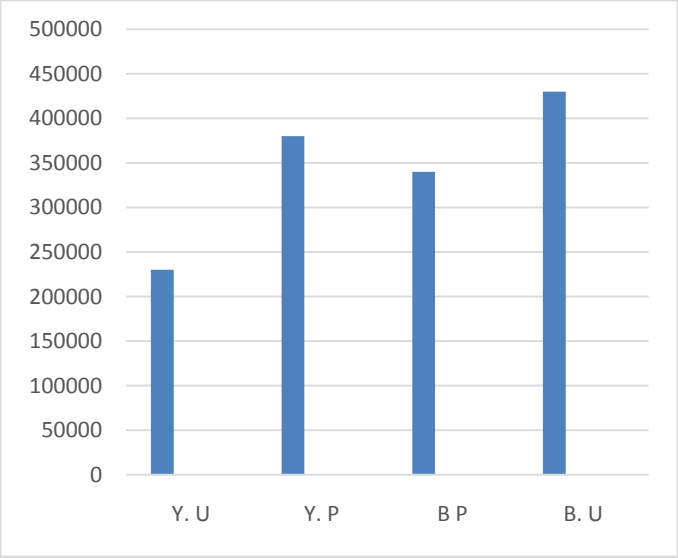

Figure-3.Total variable bacterial count after 24 hours incubation

Table-4.Total viable bacterial count after 48 hour incubation

S/N	Sample	Treatment	CFU /1ml/serial dilution
1	Yellow unpreserved	NA 10^{-3}	482 x 10^{3}
2	Yellow preserved	NA 10^{-3}	720 x 10^{3}
3	Brown preserved	NA 10^{-3}	460 x 10^{3}
4	Brwn unpreserved	NA 10^{-3}	820 x 10^{3}

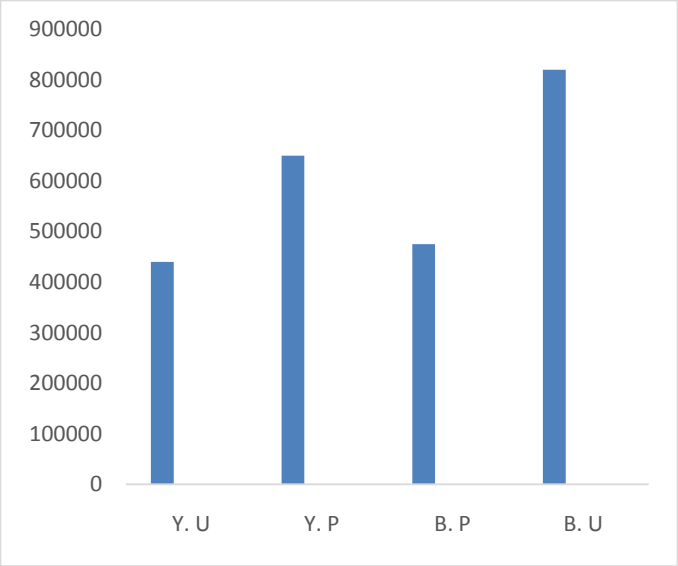

Figure-4.Total mould count at 48 hours incubation

Key:Y.U = Yellow unpreserved Y.P = Yellow Preserved B.P = Brown Preserved B.U = Brown Unpreserved

Bacterial Species Identification Gram's Staining Technique:

A drop of distilled water was placed on a clean grease slide a wire loop was sterilized over a spirit lamp flame and a small portion of the distinct colony of the bacteria were scooped out and emulsified in the drop of distilled water placed on the slide and spread out. You no air dry and fix
over the spirit flame. Flood with the crystal violet stain and add 3 drop of lugolidine and allow for 1-2 mins then wash off the stains with running water from the tap. Decolorized with acetone drop wise, then wash again with distilled water, then add the counter stain (neutral red) and allow for 1-2 min, then wash with distilled water. Place it in a slant position to drain so as it x4 magnification, and increase to higher magnification unto oil immersion (which is x100).

RESULT

Gram positive bacteria appears purple/or red in colour under the microscope, those that appears in clusters like grapes of fruits are called staphylococci and those that appear in chains are called streptococci gram negative bacteria appears bluish in colour under the microscope.

Table-5.Mean for proximate composition, pH of Preserved and Unpreserved Tigernut Milk

Sample	Protein	Carbohydrate	Fat	Crude fibre	Ash	Moisture	pH
Preserved T M	7.10	2.70	24.50	0.22	0.46	65.02	6.7
Unpreserved T M	4.50	10.0	4.30	0.20	0.66	80.34	6.34

T M = Tigernut Milk

RESULT DISCUSSIONS

The result of the proximate composition and sensory evaluation of the sample as shown in Table 5.The protein content was higher for PTM. High protein of PTM could be due to high protein content of the tiger nut. PTM is more less acidic and implies that milk prepared from it will be acceptable to patient with ulcer and other related problem since it is less acidic, the PTM is also regarded as stimulants and tonic and can be used in the treatment of indigestion, colic diarrhea, and dysentery. The pH value for UPTM also fell within the required value than UPTM. High total energy content of PTM could be probably due to high content of protein and fat the total energy value of the milk if from the fat content and hence, higher fat content is an indication of more total energies

All sample had high moisture content between 57.34 -80.34%. thus could affect the stability and safety of the ford with respect to microbial growth, and proliferation hence the products require cold storage , the milk sample had carbohydrate content between 2.7-10% and crude fiber content between 0.20-0.24%.

Table-6.Sensory evaluation scores for Preserved and Unpreserved Tigernut Milk

Sample	Colour	Aroma	Taste	Mouth feel	Overall acceptability
Preserved T M	6.85	5.45	5.8	5.3	6.2
Unpreserved T M	8.40	7.40	7.5	7.8	7.0

T M = Tigernut Milk

The sensory scores revealed various significant differences in all the parameter evaluated. Although the highest taste, aroma, colour, mouth feel and overall acceptability were recorded for preserved TM, the higher values for unpreserved TM may be due to bacterial and fungal loads.

CONCLUSION

The results revealed that milk prepared from tiger nut could be used as beverage for both the young and old person due to the high tiger nut milk nutrient contents (protein fat e.t.c) based on the sensory evaluation, the tiger nut milk were also acceptable this indicates that utilization of tiger nut will be enhanced when processed into beverage drinks. It is therefore suggested that the milk from tiger nut should be encouraged so as to solve problem of protein calorie malnutrition in Nigeria and Africa in large. The microbiological analysis of tiger nut samples. Brown preserved and brown unpreserved, Yellow preserved and Yellow unpreserved, shows that both the bacterial and fungal total loads significantly reduce in population due to the effect of the preservative used. The presence of microbe indicates inadequate aseptic technique used in the preparation process and inadequate sterilization of utensil/container used.

Among the species of the bacteria identified, the germs staphylococcuse , ecoli and yeast are pathogenic bacterial species which imply the product is not is not safe for consumption. i.e for the unpreserved Tiger

nut milk.

Further more Tiger nut is very nutritious food which serves as an excellent culture media that allow the multiplication of both bacterial and fungal species.

REFERENCES

1. The Columbia Encyclopedia Tiger nuts @ http://www.encyclopedia.com/doc/1E1-tigernut.html 2004. Accessed October, 2009.

2. Vilmorin A The Vegetable Garden. Ten Speed Press 0 ISBN 0-89815-041-8

3. Anon A Cyperules. In: The new Encyclopedia Britannica, Macropaedia Chicago. Vol.3 (15th edn), p. 185, 1992.

4. Watt K and B Breyer-Brandwijk Entry of Cyperus esculentus in Africa In: The useful plants of west tropical Africa Linn 365:373 1962.

5. Rita ES The use of tiger-nut (Cyperus esculentus), cow milk and their composite as substrates for yoghurt production. Pak. J. Nutr., 2009; 6: 755-758.

6. Consejo Regulador De Chufa de Valencia Horchata de chufas @ http://www.chufadevalencia.org 2006. Accessed December, 2009.

7. Kelley JR Biomass production of chufa (Cyperus esculentus) in a seasonally flooded Wetland. Wetlands 1990; 10:61–67.

8. James R. Kelley JR and HF Leigh Chufa Biology and Management In Waterfowl management handbook of Fish and Wildlife Leaflet 13 Washington, D.C. 1991.

9. Oderinde RA and OA Tairu Evaluation of the properties of yellow nutsedge (Cyperus esculentus) tuber oil. J. Agric. Food Chem., 1988; 28, 233-237.

10. Aliyu HN and U Sani Production of Biscuit from Composite Flour as a Substitute for Wheat. J Biosci. Res. Commun. 2009; 3: 21-27.

11. E.D. Richard and R. Ndjouenkeu, “Useful Juice Drink,” J. Food Eng.,vol. 78, no. 2, pp. 546-550, Jan. 2007.

12. H.M. Bukill, “The Useful Plants of the West Tropical Africa,” in Royal Botanic Garden, Kew, London, 2000, pp. 614-616.

13. G.D. Pamplona-Roger, “Encyclopedia of Foods and Their Healing Power,”Hong Tai Printing Co ltd., 2005, pp. 335-348.

14. R. Lancet, “Relation between consumption of sugar-sweetened drinks and childhood obesity: a prospective, observational analysis” in C.M. Brown, A.G. Dullo, and J.P. Montani, “Sugary Drinks in the Pathogenesis of Obesity and Cardiovascular Diseases,”Intl. Journal of Obesity, 2008,32, S28-S34.

15. J. Rossant, “The world's first soft drink,” Saudi Aramco World, vol. 56, no.5, pp.36-39, Sept. 2005

16. M.A. Bassiouny and J. Yang, “Influence of drinking patterns of carbonated beverages on dental erosion,” Intl. J. of Food, vol.53, pp. 33-87, 2005.

17. M. O'Hara, D. Kiefer, K. Farrell, and K. Kemper, “A review of 12 commonly used medicinal herbs,” Arch Fam Med., vol. 7, no.6, pp. 523- 536,1998.

18. NAFDAC, “Hand Book on Common Used Additive,” Lagos, Nigeria, African Publisher , 2002.

19. O.F. Owoso and O.A. Ogunmoyela, “Chemical analysis of foods; An outline,” Ibadan -Nigeria: Concept publications, 2001, pp.24-25; 55.

20. D. Pearson, “Chemical analysis of food,” 7th ed., London: Church Hill Livingstone, 1991.

21. Association of Official Analytical Chemists (AOAC). (1990). “Official methods of Analysis of the AOAC,” 15th ed. Association of Official Analytical Chemists, Arlington, Va, U.S. A. pp. 69;80.

22. Association of Official Analytical Chemists (AOAC). (1984). “Official methods of Analysis of the AOAC,” 14th ed. Association of Official Analytical Chemists, Washington, D.C., U.S. A. pp. 257-300.

23. E. Larmond, “Laboratory Methods for Sensory Evaluation of Food,”Canada Department of Agriculture, Ottawa, 1977, pp. 23-67.

24. M.O. Iwe, “Sensory methods and analysis,” Uwani – Enugu : Rojoint communication Services, 2002, pp. 34-67.

25. V. Umoh and M. Fields, “Fermentation of corn for Nigerian agidi,”Journal of Food Science, Vol. 46, no. 3, pp. 903–905, May 1981.