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Determination of amino acids and physico-chemical properties of juice samples produced from five varieties of tigernut (*Cyperus esculentus*)

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Abstract Production of tigernut juice from four varieties of tigernut (*Tiger-Var01*, *Tiger-Var02*, *Tiger-Var03* and *Tiger-Var04*) was carried out in this study, with the aim of analysing some of its chemical properties and amino acids. The organoleptic properties of the juice samples were also investigated. The pH of the juice samples ranged between 6.57 and 6.61, with the *Tiger-Var01* sample having the highest value. Juice sample from *Tiger-Var03* recorded higher ash content (%) of 0.39 than others while *Tiger-Var04* had the highest protein of 2.51. There was no significant difference (p>0.05) between most of the chemical properties of the juice samples. Microbial analysis showed that counts (log cfu/ml) of between 3.45 and 4.27 were recorded for the juice samples, with *Tiger-Var04* having the highest count. Yeast and mould counts were below 2.6 while counts of coliforms and staphylococci were below 2.0. Glutamic acid was present in higher concentrations (> 10 g/16 g N) than others in all the tigernut juice samples. Other amino acids that were present in considerable quantities include aspartic acid, arginine, leucine and serine, each of which occurred at levels higher than 3 g/16 g N in all samples. Essential amino acids such as leucine, isoleucine, threonine and valine were recorded in the tigernut juice samples, suggesting that tigernut could offer some amino acids needed by the body. Mean scores were higher than 5.0 in all the organoleptic attributes tested. It was concluded that acceptable juice could be produced from the four tigernut varieties.

Keywords tigernut juice; amino acids; chemical properties; organoleptic properties;microbial analysis

Introduction

Tiger-nut (*Cyperus esculentus L.*) belongs to the Division–*Magnoliophyta*, Class–*Liliopsida*, Order–*cyperales* and Family–*Cyperaceae* and was found to be a cosmopolitan, perennial crop of the same genus as the papyrus plant [1]. The tubers are about the size of peanuts and are abundantly produced in Nigeria. It has many other names like Zulu nut, yellow nut grass, ground almond, chufa, edible rush and rush nut**.** In Nigeria, the Hausas call it "Aya", Yorubas "imumu", the Igbos "aki Hausa", "ofio" in southern Nigeria. Tiger-nut has been cultivated since early times (chiefly in south Europe and West Africa) for its small tuberous rhizomes which are eaten raw or roasted, used as hog feed or pressed for its juice to make a beverage. Tiger nut, an under-utilized crop, was reported to be high in dietary fiber content, which could be effective in the treatment and prevention of many diseases including colon cancer, coronary heart diseases, obesity, diabetes and gastrointestinal diseases [1]. It has 5.8% moisture, rich in protein (7%) and carbohydrate such as reducing sugar (7.4%), soluble polysaccharide (7.4%) and starch (86.4%).

The protein in tiger nut is of high biological value considering the many essential amino acids it contains. These amino acids are high and can satisfy amino acid need of adults. The nuts are valued for their highly nutritious starch content, dietary fiber and carbohydrate, including mono, di and polysaccharides [2]. The nut was reported to be rich in sucrose (17.4 to 20.0%), fat (25.50%), and protein (8%). The nut is also very rich in mineral content such as sodium, calcium, potassium, magnesium, zinc and traces of copper. It has been shown 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 solubleand 12% soluble (Muhammad *et al.*, 2011). The nuts are said to the best imulant and tonic and also used in the treatment of indigestion, colic diarrhea, dysentery and excessive thirst. Tigernut juice (otherwise known as tigernut milk) is a refreshing purely natural vegetable drink and/ or dessert, which is prepared with water, sugar and tiger-nuts. It is a very nutritive, energy drink both for young and old. The qualities of Tiger-nut (*Cyperusesculentus*) in this context stimulate its inclusion in the preparation of beverage so as to provide protein energy-rich drink at affordable price in place of animal protein/fat which is scarce and expensive.

Tigernuts are cultivated throughout the world including Nigeria, especially in the northern part, and other West Africa Countries like Guinea, Cote d'ivore, Cameroon, Senegal, America and other parts of the World [3]. The nut has also been reported to be rich in sucrose (17.4–20.0%), fat (25.50%), protein (8%), and minerals such as sodium, calcium, potassium, and magnesium [4].

Though studies have been carried out on the production of tigernut juice by many researchers [1-2, 5], however the amino acid profiles of the juice have not been reported. The present study was aimed at producing juice extract from tigernut, with the view of analyzing some of its chemical properties and amino acids. This could further help educate consumers on some of nutritional benefits associated with tigernut juice.

Materials and Methods

Sources of raw materials

The tigernut varieties used in this study, coded as *Tiger-Var01*, *Tiger-Var02, Tiger-Var03* and *Tiger-Var04,* were obtained from alocal market in Offa, Kwara State, Nigeria. The lecithin used as stabiliser was purchased from Sigma Aldrich Chemicals, UK.

Production of tigernut juice

Juice was extracted from the tigernut varieties using the method described by Jiang *et al.* (2013) with modification [6]. A kilogram (1 kg) of healthy tigernuts was measured and washed with clean tap water. They were then soaked in 3 litre of clean water (1:3% w/v) for 10 h, after which they were drained. The drained nuts were mixed with water (1:5% w/v) and blended using aa electric blender [2]. The resulting homogenous slurry was filtered using a muslin cloth and the resultant filterate was tigernut juice (Figure 1).The juice samples were pasteurized at 67 ^{o}C for 30 minutes prior to commencement of analysis.

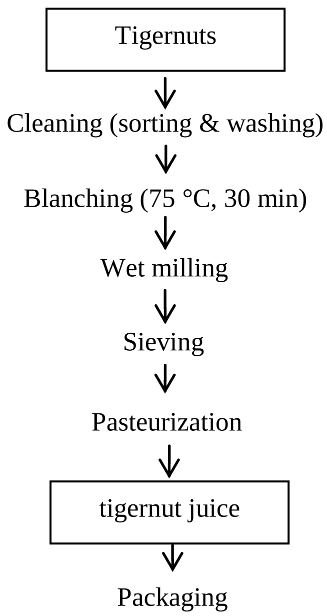

Figure 1: Flow chart showing production of tigernut juice

Determination of chemical properties in the tigernut juice samples

The chemical properties of tigernut juice samples, including crude fiber, ash, fat, protein and moisture content were determined using the methods of Association of Official Analytical Chemists [7]. Carbohydrate was determined by difference.

Titratable acidity was also determined by the method of AOAC 2005 [7], while pH was measured by a pH meter (pH 212 Microprocessor, Hanna Instruments, USA) using the reported method [8].

Determination of amino acid contentsin the tigernut juice samples

The amino acid contents of the different tigernut juice samples were determined using the method described by Wang and Cavins (1989) [9], with little modifications where necessary. Tigernut juice was defatted, dried and then hydrolyzed for 24 h by refluxing in 6N hydrochloric acid, evaporated to dryness, and dissolved in citrate buffer (pH 2.2). A portion of the hydrolysate with norleucine as internal standard was analyzed for amino acids with a Trace GC Ultra gas chromatograph (Thermo Electron Corporation) system which automatically computed the resulting data.

Microbial enumeration of the tigernut juice samples

The lactic acid bacteria (LAB), yeasts and moulds (Y&M), total bacteria (TB), coliforms, micrococci, staphylococci and enterobacteriaceae, counts were determined in the tigernut juice samples using the methods described by Olaoye and Dodd (2010) [10]. LAB, enumerated on deManRogossa Sharpe (MRS, Oxoid, UK); Y&M onsabouraud agar (Oxoid, UK); TB on plate count agar (PCA, Sigma Aldrich, UK); coliforms on mackonkey agar (Oxoid, UK); micrococci and staphylococci on mannitol salt agar (Oxoid, UK); and enterobacteriaceae on violet red bile glucose agar (Sigma Aldrich, UK). All media were incubated at 30 °C for 24 h, except Y & M (25 ^{o}C for 72 h). Colonies on the different media were counted and results expressed in logarithm of colony forming unit per ml of tigernut juice (log CFU/ml).

Analysis of organoleptic attributes of tigernut juice samples

The different tigernut juice samples were subjected to sensory evaluation immediately after preparation. They were evaluated for the attributes of colour, mouth feel, aroma, taste and general acceptability using a 50 member panel; members were requested to allocate scores to the samples using a 9-point hedonic scale (from 1-dislike extremely to 9-like extremely). The data obtained were subjected to statistical analysis.

Statistical analysis

The data obtained, which depended on the soybean varieties were analyzed using the means of three replicates of samples. Means were separated and analysed using the *t*-test in data analysis functionality of Microsoft Excel 2010 to determine differences. Significant differences between samples were determined at $P<0.05$.

Results and Discussion

The chemical properties of the tigernut juice samples from the different varieties of tigernut are shown in Table 1. pH values ranged from 6.57 to 6.61, with juice made from tigernut variety *Tiger-Var04* having the lowest while that from *Tiger-Var03* had the highest. Crude fiber was lowest (0.23%) for juice from *Tiger-Var01*, but the highest value of 0.31 was recorded for that from *Tiger-Var03*. The ash contents (%) recorded for the tigernut juice samples ranged between 0.31 and 0.39, with that of the *Tiger-Var03* variety recording the highest value. A value of 1.59% was obtained for sample from *Tiger-Var02*as the highest fat content while the lowest value of 1.26 was recorded for sample from the *Tiger-Var04* variety. Protein contents (%) ranged from 2.34 and 2.51 while carbohydrate of between 1.93 and 2.34 were recorded for the tigernut juice samples. The sample from *Tiger-Var02* had the highest titratable acidity of 0.20 (% lactic acid) while the lowest value (0.18) was recorded for that of *Tiger-Var01*. The pH, carbohydrate, crude fiber and ash contents of the tigernut juice samples recorded in this study were similar to those reported [1]. There was however difference in the values recorded by the researcher in terms of protein and moisture in comparison with those obtained in the present study. The difference could be attributed to genetic variation in the varieties of tigernut used in the respective studies as well as methods adopted for preparation of the juices [11]. In a related study, Ukwuru*et al.* (2011) also reported values of protein and crude fiber in tigernut beverage (juice), similar to those recorded in this present study [5]. Titratable acidity of the tigernut juice samples was generally low while their pH remained within neutral range. This observation may be attributed to the fact that the samples did not undergo fermentation that could otherwise reduce their pH, and hence increase titratable acidity.

Presented in Table 2 are the counts of different microorganisms (log cfu/g) associated with the tigernut juice samples. The highest count (4.27) of lactic acid bacteria was recorded for the *Tiger-Var04* sample and the lowest count (3.47) for *Tiger-Var01*. Counts of yeast and moulds were between 1.93 and 2.47 for all samples, and this was supportive of the report of Ukwuru*et al.* (2011) who recorded similar results from a beverage made from mainly tigernut [5]. Counts of total bacteria indicated that the lowest value (5.29) was obtained for the *Tiger-Var03* sample while the highest count of 5.93 was recorded for *Tiger-Var01*. The counts of coliforms and staphylococci in the tigernut juice samples were lower (<2) than those recorded for other microorganisms; this could be very advantageous as a result of public health significance of coliforms and staphylococci. The presence of the organisms, especially in unacceptable level, has been observed to be undesirable [12], besides they can also contribute to acquisition of food borne diseases [13-14]. Micrococci counts ranged between 1.94 and 2.34 while a range of 2.59 and 2.82 was recorded for enterobacteriaceae. Generally, there was no significant difference (p>0.05) between all the samples in the counts of different microorganisms that were enumerated.

The different amino acids (g/16 g N) in the tigernut juice samples are shown in Table 3. Seventeen (17) amino acids were detected and quantified in the samples, some of which belong to the essential group of amino acids required for human nutrition. Glutamic acid was present in higher concentrations (> 10 g/16 g N) than others in all the tigernut juice samples. Other amino acids that were present in considerable quantities include aspartic acid, arginine, leucine and serine, each of which occurred at levels higher than 3 g/16 g N in all samples. A similar observation was reported by Nurliyani and Sunarti (2014) in a related study [15]. The different types of amino acids observed in the soymilk samples in the present study may imply that useful proteins that are required by human could be provided by soymilk when incorporated into human diet. The tigernut juice samples contained essential amino acids such as leucine, isoleucine, threonine and valine, suggesting that tigernut could offer some amino acids needed by the body, and which it may not be able to synthesize [16]. The varieties of tigernut used in this study, when processed into juice, may be useful in promoting availability of vegetable beverages of nutritional benefit to consumers in Nigeria, especially among majority of the populace who could not afford beverages from commercial sources for economic reasons.

Mean scores of the organoleptic evaluation of the different tigernut juice samples (Table 4) indicate that the *Tiger-Var02* sample had the highest mean score (7.6) for the organoleptic attribute of colour while the lowest score of 5.7 was obtained for *Tiger-Var04*. Scores ranged between 5.4 and 6.7 for the attribute of mouth feel, with *Tiger-Var01*and *Tiger-Var03*having the lowest and highest values respectively. Mean scores of 6.7, 6.0, 5.5 and 5.0 were recorded for juice samples from respective *Tiger-Var01*, *Tiger-Var02*, *Tiger-Var03* and *Tiger-Var04* varieties in the attribute of aroma. In terms of the organoleptic attribute of taste, the *Tiger-Var01* sample had the highest mean score of 7.2 while the lowest score (5.1) was recorded for the juice sample made from *Tiger-Var03*. The mean scores of generally acceptability show that the *Tiger-Var01* sample was most preferred by panel lists, having the highest score of 7.2, while sample from *Tiger-Var03* was least preferred as it had the lowest score of 6.2. It is however interesting to note that no significant difference (p>0.05) was recorded among the different tigernut juice samples in most of the organoleptic attributes that were evaluated in the course of the present study. This implies good and acceptable juice samples could be obtained from all the four tigernut varieties.

Table 1: Chemical properties of the tigernut juice samples

Properties	***Tiger-Var01***		***Tiger-Var02***		***Tiger-Var03***		***Tiger-Var04***	
	value	**SD**	***value***	**SD**	***value***	**SD**	***value***	**SD**
pH	6.61	0.47	6.59	0.93	6.62	1.02	6.57	0.66
Crude fibre (%)	0.23	0.09	0.26	0.11	0.31	0.08	0.26	0.03
Ash (%)	0.36	0.05	0.31	0.08	0.39	0.13	0.35	0.05
Fat (%)	1.54	0.92	1.59	0.74	1.37	0.36	1.26	0.62
Protein (%)	2.34	0.27	2.34	0.95	2.37	0.66	2.51	0.83
Carbohydrate (%)	2.34	0.52	1.93	0.82	2.1	0.74	1.99	0.75
Moisture content (%)	92.19	5.48	93.16	10.16	93.45	8.29	93.61	6.27
Titratable acidity (% lactic acid)	0.18	0.01	0.2	0.01	0.19	0.02	0.19	0.03

Each value is an average three replicates; Figures having same superscript letters across rows are not significantly different (P>0.05); *Tiger-Var01*, Evaporated juice from *tigernut* variety 01*; Tiger-Var02*, Evaporated juice from *tigernut* variety 02*; Tiger-Var03*, Evaporated juice from *tigernut* variety 03; *Tiger-Var04,* Evaporated juice from *tigernut* variety 04; SD, standard deviation.

Table 2: Counts (log cfu/ml) of different microorganisms in the tigernut juice samples

Microorganisms	*Tiger-Var01*		*Tiger-Var02*		*Tiger-Var03*		*Tiger-Var04*	
	value	**SD**	*value*	**SD**	*value*	**SD**	*value*	**SD**
Lactic acid bacteria	3.45	0.92	4.01	1.29	3.92	0.74	4.27	1.04
Yeast & moulds	1.96	0.94	2.31	0.86	2.47	0.37	1.93	0.66
Total bacteria count	5.93	1.29	5.72	0.94	5.29	1.29	5.38	0.57
Coliforms	1.2	0.08	1.16	0.06	1.17	0.19	1.17	0.22
Micrococci	2.17	0.43	2.33	0.27	1.92	0.14	2.34	1.02
Staphylococci	1.43	0.34	1.63	0.39	1.68	0.31	1.83	0.33
Enterobacteriaceae	2.59	0.28	2.82	1.02	2.67	0.94	2.62	0.03

Each value is an average three replicates; Figures having same superscript letters across rows are not significantly different (P>0.05); *Tiger-Var01*, Evaporated juice from *tigernut* variety 01*; Tiger-Var02,* Evaporated juice from *tigernut* variety 02*; Tiger-Var03,* Evaporated juice from *tigernut* variety 03; *Tiger-Var04,* Evaporated juice from *tigernut* variety 04; SD, standard deviation.

Table 3 Concentrations of amino acids (g/16 g N) in the tigernut juice samples

Amino acids	*Tiger-Var01*		*Tiger-Var02*		*Tiger-Var03*		*Tiger-Var04*	
	value	**SD**	*value*	**SD**	*value*	**SD**	*value*	**SD**
Lysine	2.87	0.51	3.31	0.38	2.99	1.09	3.19	0.55
Histidine	1.72	0.64	1.02	0.47	1.18	0.36	1.26	0.72
Aspartic acid	5.96	0.93	5.68	1.21	6.95	1.28	6.2	1.62
Threonine	2.19	0.72	3.02	1.02	2.51	0.86	3.01	1.28
Arginine	4.67	0.94	4.19	0.88	3.69	1.29	3.75	0.95
Phenylalanine	2.79	0.44	3.29	1.34	2.48	0.84	2.73	1.2
Valine	3.23	1.28	2.36	1.05	2.36	1.06	3.27	1.09
Cystine	1.01	0.35	0.83	0.26	0.94	0.33	0.74	0.05
Methionine	1.04	0.06	0.93	0.25	1.28	0.57	0.85	0.77
Tyrosine	2.97	0.72	2.1	0.43	2.73	0.73	1.92	0.48
Proline	2.89	1.02	3.46	1.25	3.44	0.56	3.27	1.64
Glycine	2.1	0.84	2.35	0.46	2.93	0.73	2.14	0.73
Alanine	3.28	1.29	3.2	0.86	3.91	1.02	2.97	0.93
Isoleucine	3.2	0.78	2.99	0.84	3.02	0.83	2.38	0.47
Leucine	5.48	0.97	5.2	1.36	6.57	1.73	5.49	1.74
Serine	3.29	0.84	3.28	0.77	3.23	1.02	3.27	0.76
Glutamic acid	11.28	2.16	10.27	3.25	13.26	4.36	11.92	4.36

Each value is an average three replicates; Figures having same superscript letters across rows are not significantly different (P>0.05); *Tiger-Var01*, Evaporated juice from *tigernut* variety 01*; Tiger-Var02,* Evaporated juice from *tigernut* variety 02*; Tiger-Var03*, Evaporated juice from *tigernut* variety 03; *Tiger-Var04,* Evaporated juice from *tigernut* variety 04; SD, standard deviation

Table 4: Organoleptic mean scores of the tigernut juice samples

Samples	***Attributes***				
	Colour	**Mouthfeel**	**Aroma**	**Taste**	**GenAccep**
Tiger-Var01	7.3±1.2	5.4±0.92	6.7±2.1	7.2±2.1	7.2±2.7
Tiger-Var02	7.6±2.8	6.4±1.7	6.0±2.1	5.9±0.8	6.9±2.5
Tiger-Var03	5.7±3.2	6.7±0.8	5.5±1.5	5.1±0.9	6.2±1.2
Tiger-Var04	6.0±0.6	6.5±2.3	5.0±0.6	5.8±2.0	6.3±1.8

Each value is an average three replicates; Figures having same superscript letters across rows are not significantly different (P>0.05); *Tiger-Var01*, Evaporated juice from *tigernut* variety 01*; Tiger-Var02,* Evaporated juice from *tigernut* variety 02*; Tiger-Var03,* Evaporated juice from *tigernut* variety 03; *Tiger-Var04,* Evaporated juice from *tigernut* variety 04; SD, standard deviation.

Based on the results of this finding, it could be concluded that the tigernut juice samples had considerable content of ash and protein, which may play important role towards alleviating malnutrition among consumers, if production of the product is promoted in Nigeria. Additionally, utilization of locally available vegetables would be enhanced when necessary and favourable government policies are encouraged. This would be a source of encouragement to local farmers in increasing their earnings. Local food processors and consumers also stand to benefit immensely in saving foreign exchange normally associated with importation of juice drinks from offshore countries. Of most important is the nutritional advantage inherent in the processing of local fruits and vegetables in the production of juice drinks for consumers in developing countries of the world, with Nigeria as typical example.

References

1. Udeozor, L.O. (2012). Tigernut-soy milk drink: preparation, proximate composition and sensory qualities. *International Journal of Food and Nutrition Science*, 1: 18-26.
2. Olaoye, O.A., Ubbor, S.C., & Uduma, E.A. (2016). Determination of vitamins, minerals and microbial loads of fortified non-alcoholic beverage (*kununzaki*) produced from millet. *Food Science and Nutrition*, 4: 96–102. doi: 10.1002/fsn3.267.
3. Belewu, M.A., & Abodunrin, O.A. (2006). Preparation of Kunnu from unexploited rich food source: tiger nut (*Cyperus esculentus*). *World Journal of Dairy and Food Science*, 1:19-21.
4. Umerie, S.C., & Enebeli, J.N. (1997). Malt caramel from the nuts of *Cyperusesculentus*. *Journal of Biological and Resource Technology*, 8:215-216.
5. Ukwuru, M.U., Ibeneme, C.L., Agbo, G.I. (2011). New product development from tigernut (*cyperus esculentus*) and their sensory, proximate and microbiological evaluation. *Pakistan Journal of Nutrition*, 10: 101-105.
6. Jiang, S., Weixi, C., &Baojun, X. (2013). Food quality improvement of soy milk Made from Short-Time Germinated Soybeans. *Foods*, 2: 198-212.
7. AOAC, Association of Official Analytical Chemists. (2005). Official methods of analysis of the Association of Analytical Chemists International.18th ed. AOAC, Gaithersburg, MD.
8. Marugg, J.D., Gonzalez, C.F., Kunka, B.S., Ledeboer, A.M., Pucci, M.J., Toonen, M.Y., Walker, S.A., Zoetmulder, L.C.M., &Vandenbergh, P.A. (1992).Cloning, Expression, and Nucleotide Sequence of Genes Involved in Production of Pediocin PA-i, a Bacteriocin from *Pediococcusacidilactici*PAC1.O.*Applied and Environmental Microbiology*, 58: 2360-2367.
9. Wang, H.L., &Cavins, J.F. (1989). Yield and amino acid composition of fractions obtained during tofu production. *Cereal Chemistry*, 66: 359-361.
10. Olaoye, O.A., &Dodd, C.E.R. (2010). Evaluation of bacteriocinogenic*Pediococcusacidilactici* as protective culture in the preservation of *tsire*, a traditional Nigerian stick meat.*Journal of Food Safety*, 30: 867-888.
11. Olaoye, O.A. (2015). A study on quality parameters and shelf stability of sweetened condensed vegetable milks produced from four varieties of soybeans (Glycine max). *International Food Research Journal*, 2: 77-84.
12. Olaoye, O.A., & Onilude, A.A. (2009). Assessment of microbiological quality of sachet packaged drinking water in Western Nigeria and its public health significance. *Public Health*, 123: 729-734.
13. Olaoye, O.A., & Onilude, A.A. (2010).Investigation on the potential use of biological agents in the extension of fresh beef in Nigeria. *World Journal of Microbiology and Biotechnology*, 26: 1445-1454.
14. Olaoye, O.A., Onilude, A.A., & Idowu, O.A. (2011). Microbiological profile of goat meat inoculated with lactic acid bacteria cultures and stored at 30°C for 7 days. *Food and Bioprocess Technology*, 4: 312-319.
15. Nurliyani, E.H., & Sunarti (2014). Microbiological quality, fatty acid and amino acid profile of kefir produced from combination of goat and soymilk. *Pakistan Journal of Nutrition*, 13: 107-115.
16. Hajirostamloo, B., & Mahastie, P. (2008).Composition of soymilk and cowmilk nutritional parameter. *Research Journal of Biological Sciences*, 3: 1324-1326.