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Evaluation of Amino Acids in the Milk Preparations from Tiger Nut Rhizome and Its Mixed Varieties

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Abstract: The amino acid composition of yellow and brown tiger nut (*Cyperus esculentus*) milk preparations and their mixtures were studied. The mixed variety was prepared from mixing equal proportion of the two varieties. The milk preparation was obtained by blending and subsequent expression of the milk. Protein analysis stated with the hydrolysis of the milk preparation with HCl for 23hrs and the resulting amino acids were quantified with amino acid analyzer (Technicom TSM-1). The % nitrogen for the yellow, Brown and the mixture are 3.72, 3.24 and 2.22 respectively. Arginine has the highest value of 15.87, 16.22 and 19.20 g/100g respectively conferring same order of amino acid basicity. Methionine and tyrosine are the lowest (0.60 and 0.63, 0.68 and 0.48, 0.49 and 0.48 g/100g protein) respectively. Tryptophan is not prominently associated with the myriad of essential amino acids of tiger nut milk. It was not detectable in any of the varieties. The overall result of the study showed that the tiger nut milk is a rich source of protein. It can also be consumed as a valuable substitute for conventional cow milk and other known milk sources. The milk prepared from mixed variety exhibited synergistic effect as it combined some beneficial amino acid that either of the two varieties lacks.

Keywords : Tiger nut, milk, amino acid, synergistic effect, protein

I. INTRODUCTION

Tiger-nuts (*Cyperus esculentus L.*) are member of the division–*Magnoliophyta*, class*liliopsida*, order –*cyperales* and family. Tiger nuts are also known as “earth almond”, “chufa” and “Zulu nut”. Locally in Nigeria, it is called *Ayaya*, *Ofo* and *Akiuasa* in Hausa, Yoruba and Igbo languages where the three varieties (black, brown and yellow) are cultivated. The yellow specie are bigger in size, bright yellow colour, smooth body, yields more milky liquid upon expression [1]. People consume tiger nut in raw, baked or roasted form or in form of beverage referred to as tiger nut milk. It also finds uses as a flavoring agent for ice cream and biscuit, as well in making oil, soap, starch and flour [2] and [3].

Amino acids are the hydrolysed units of proteins (also known as polypeptides). Amino acid units in protein are arranged in a linear chain usually folded into a globular form joined together by the peptide bonds between the carboxyl and amino groups of adjacent amino acid residues. Proteins are also necessary in animals' diets, since animals cannot synthesize all the amino acids they need and must obtain essential amino acids from food. Through the process of digestion, animals break down ingested protein into free amino acids that are then used in metabolism. Thus, they function as the protein building blocks and part of as well as co-enzymatic action.

Milk preparation is commonly used for any consumable white opaque liquid that resembles the liquid produced from the mammary glands of mammals. The terms: soya milk, rice milk, almond milk, and coconut milk are used to depict the appearance of whitish fluid expressed from them. Since these *milks* are also found to be nutritious, they are acceptable as non-animal milk substitutes.

Milk is an invaluable source of paying protein nutritional deficit. Cow milk has been the conventional animal source. Tiger nut rhizome milk preparation is an under-explored plant source. This study is therefore aimed at quantifying the protein content of the milk preparations of tiger nut rhizome varieties and their mixture. Furthermore, the study is to assess the amino acid content, classes and its protein quality scoring relative to the global human protein requirement standard.

II. MATERIALS AND METHODS

Collection of samples

Samples of brown and yellow variety tiger nut rhizome were bought in Minna central market, Niger State, Nigeria. The samples were spread on a flat tray so as to remove the stones, sands and other particles in the

tiger nut. The samples are poured in separate basins of water and washed to further remove fine sand and dust from the body of the samples.

Preparation of milk from tiger nut rhizome

The yellow and brown tiger nut rhizome were blended separately in a warring blender for 5-6 minutes and a 100g of the blended sample was weighed on the weighing balance, the weighed sample was poured in a 400cm³ beaker. 200cm³ of distilled water was then poured in it. The constituent was thoroughly mixed together and filtered using a muslin cloth. The prepared milk was refrigerated for preservation.

A 50g each of both samples was weighed on the weighing balance and was poured in a 400 cm³ beaker. 200 cm³ of distilled water was then poured into the container. This was thoroughly mashed, blended and homogenized. Muslin cloth was used in filtering to extract the milk. The extracted milk was refrigerated for preservation pending the next stage.

Reagent

The reagent used for the hydrolysis of the protein in the tiger nut samples include: Ninhydrin, Hydrazine Sulphate, 50% Methyl Cellulose, 0.2mol.dm⁻³ NaOH. Buffer pH 3.25, Buffer pH 4.25, Buffer pH 5.25. Technicom Amino Acid analyzer (TSM Sequential Multi sample Analyzer) was used as the major equipment.

Hydrolysis of sample

The sample was placed in a hydrolysis tube and left until was dried using rotary evaporator. The expunged water is to avoid the dilution of the hydrolyzing acid. 10cm³ of Hydrolysis Solution was added per 0.05mg of lyophilized protein. The sample tubes were frozen in a dry ice-acetone bath, and flame sealed in vacuum. Hydrolysis of sample was carried out at 110°C for 23 hours with 0.01mol. dm⁻³ HCl. This was in a vacuum with inert atmospheric condition to prevent oxidation. Extended hydrolysis time of 48 and 72 hours were considered for a complete hydrolysis of protein.

Determination of amino acid

The filler plug was pulled out from the cartridge of the analyzer and then filled with 0.20 mol.dm⁻³ NaOH - hydrolysate and 0.03 mol.dm⁻³ LiOH-physiological media. Using, 1cm³ of the liquid reagent, the mixture was forced through the cartridge using a 1cm³ plastic tuberculin syringe. The cartridge was emptied by inverting and shaking to remove all liquid. A pH 2.0 buffer -0.20 mol.dm⁻³ sodium citrate-hydrolysate; 0.30 mol.dm⁻³ Lithium citrate-physiological media was replaced in the cartridge. With the cartridge empty and the resin equilibrated at pH 2.0, the sample was loaded into the cartridge. Vacuum was applied to the bottom of the cartridge while in vertical position. This continued until the cartridge was drawn to the top of the filler. The filler tray was replaced and the cartridge is then placed on the sample tray. The filler tray and the inner cartridge were handled using hand gloves rather than bare hand. This was done to avoid contamination through the skin amino acids.

III. RESULTS AND DISCUSSION (11 BOLD)

Amino Acid Content

The essential or indispensable amino acids can only be obtained from foods. This is because human body is not able to get them at the level needed for normal growth [4]. Arginine has the highest concentration of 15.87, 16.22, 19.20 (g/100g) respectively for the yellow, brown and mixed tiger nuts respectively (Table 1). Phenylalanine partnered with tyrosine had the concentration of 3.71, 3.73 and 3.39g/100g protein respectively. Methionine and Tyrosine of the three samples (yellow, brown and mixed) are the lowest, with the respective values of (0.60 and 0.63; 0.68 and 0.48; 0.49 and 0.48 g/100g protein).

The values were comparable with the [5] human protein requirement. Tryptophan amino acid was not detected with the method used. This deficiency does not have pronounced effect as its adult human requirement is quite as low as 4mg/Kg/day. This value is equivalent to 0.24g needed by adult of average 60 kg body weight. This shows that the tiger nut varieties' milk and their mixture as protein rich food. This is as result of its close protein content values with the human protein requirement as recommended by [6] and [5].

Furthermore, leucine and threonine in table 1 indicate percentages relative to standard requirements as 162 and 202. These values are comparable to those of leucine and threonine found in Egg, beef and milk [5] and quite higher than the respective value of amino acid distribution in average Indian diet [7]. Arginine in tiger nut milk 10.31 in table 1 is also close to the value obtained and reported by [8] for Piper guineense which is known to be of high protein content.

The total amino acid content encompasses the essential and non-essential amino acids. The values reflect the total protein present in the tiger nut varieties. The value obtained showed that the tiger nut milk studied could well satisfy the standard human protein requirements as reported by [9].

Amino Acid Classification

The evaluation report on the amino acid based on classification is shown in Table 2. The total amino acid for the yellow tiger nut milk, Brown tiger nut milk and its mixture, TAA (53.39, 53.61 and 50.85 g/100g protein), these values are within the range of the value reported in plant foods which range between 39.3-76.5g/100g protein [10]. The Total Sulphur Amino Acid in the three samples are (2.72, 2.47 and 2.34 g/100g) which are comparable to the values recommended for young men [11]. Same comparable values were observed for the Essential Aromatic Amino Acid. Table 2 also revealed that the amino acids in the studied tiger nut varieties are more basic in nature as the Total Acidic Amino Acid, TAAA which was found to be lower than the Total Basic Amino Acid. The high concentration of Arginine, an essential amino acid, accounts for larger amount of this basic nature of the preparation.

Amino Acid Scoring

The dietary protein quality [6] and [12] is expressed as the ratio of the amino acid available in the protein to its needs:

$$\text{Amino acid score (AMS)} = \frac{100 \times \text{mg of amino acid per g test protein}}{\text{mg of amino acid per g reference protein}}$$

Amino acid scoring predicts the degree of efficiency that a sourced protein will meet the amino acid needs of a person or group. The amino acid scoring table (Table 3) shows that in the yellow tiger nut milk, the first limiting amino acid is Leucine with value of 0.15, followed by Isoleucine as the second limiting amino acid with 0.31. The brown tiger nut milk, its first limiting amino acid is Leucine with the value 0.16 and Isoleucine as the second limiting amino acid with the value 0.30, in the mixture of the milk the first limiting amino acid is Leucine with the value 0.25 and Lysine as the second limiting amino acid with value 0.32. It is also observed that the values of yellow and brown tiger nut milk are comparable. The patterns of the limiting amino acids simply suggest that the affected essential amino acids were present at lower degree than predicted but not actually missing. Based on the values, the protein quality of the tiger nut varieties' milk is not far from predicted standard.

IV. FIGURES AND TABLES

Table 1: Amino acids content of the milk prepared from the tiger nut rhizomes (g/100g crude protein)

Amino acid	Yellow	Brown	Mixed	APR(g)
	<u>Essential Amino Acids (EAA)</u>			
Arginine (Arg)	15.87	16.22	19.20	-
Histidine (His)	1.64	1.70	1.39	0.90
Isoleucine (Ile)	1.24	1.14	1.33	1.20
Leucine (Leu)	1.04	1.15	1.91	2.34
Lysine (Lys)	2.92	2.29	1.78	1.80
Methionine (Met)	0.60	0.68	0.49	0.60
Phenylalanine (Phe)	3.08	3.25	2.91	6.84
Threonine (Thr)	1.82	1.71	1.79	0.90
Tyrosine (Tys)	0.63	0.48	0.48	0.66
Valine (Val)	1.99	2.34	1.73	1.56
	<u>Non- Essential Amino Acids (NEAA)</u>			
Alanine (Ala)	2.58	3.00	2.05	
Aspartic acid (Asp)	4.31	4.00	3.01	
Cystine (Cys)	2.12	1.79	1.85	
Glutamic acid (Glu)	6.28	6.59	4.54	
Glycine (Gly)	3.41	3.55	3.03	
Proline (Pro)	1.83	1.93	1.53	
Serine (Ser)	2.03	1.79	1.83	
Total Amino Acid(TAA)	53.39	53.61	50.85	

Table 2: Classification of Essential, Non-essential, Acidic, Basic and Aromatic Amino Acid (g/100g protein) of tiger nut rhizome milk.

Classification		Yellow	Brown	Mixture
1	Total Amino Acid (TAA)	53.39	53.61	50.85
2	Total Essential Amino Acid (TEAA)			
	With Histidine	30.83	30.96	30.01
	Without Histidine	29.19	29.26	31.62
3	% TEAA			
	With Histidine	57.70	57.70	59.00
	Without Histidine	54.60	54.60	62.20
4	Total Non-Essential Amino Acid (TNEAA)	22.56	22.65	17.84
	%TNEAA	42.20	42.20	35.00
5	Essential Aliphatic Amino Acid (EAAA)	4.27	4.63	4.97
	%EAAA	8.00	8.60	9.77
6	Essential Aromatic Amino Acid (EArAA)	3.71	3.73	3.39
	% EArAA	6.90	6.95	6.66
7	Total Acidic Amino Acid (TAAA)	10.59	10.59	7.55
	%TAAA	19.80	19.80	14.80
8	Total Basic Amino Acid (TBAA)	20.43	20.21	22.37
	%TBAA	38.20	37.70	44
9	Total Sulphur Amino Acid (TSAA)	2.72	2.47	2.34
	%TSAA	5.09	4.60	4.60
	% Cysteine in TSAA	77.90	72.50	79.00

Table 3: Amino acid scores of tiger nut rhizome milk

Amino acids	Yellow			Brown		Mixture	
	*PAAESPa	EAAC	AMS	EAAC	AMS	EAAC	AMS
Ile	4.00	1.24	0.31	1.14	0.30	1.33	0.33
Leu	7.00	1.04	0.15	1.15	0.16	1.91	0.27
Lys	5.50	2.92	0.53	2.29	0.42	1.78	0.32
Met + Cys	3.50	2.72	0.78	2.47	0.71	2.34	0.67
Phe + Tys	6.00	3.71	0.62	3.73	0.62	3.39	0.57
Thr	4.00	1.82	0.46	1.71	0.43	1.79	0.45
Try	1.00	nd	na	nd	na	nd	Na
Val	5.00	1.99	0.40	2.34	0.47	1.73	0.35
Total	36.00	15.44	3.25	14.83	3.11	14.27	2.96

*: [13] PAAESPa = provisional Amino Acid (Egg) scoring Pattern; EAAC = Essential Amino Acid Composition (see Table 1); AMS = Amino Acid Scores; nd = not determined; na = not available.

V. CONCLUSION

The results obtained in protein analysis of the tiger nut varieties' milk prepared from tiger nut rhizome falls within the FAO/WHO/UNU requirement for both infant and adult. This relatively, under explored milk preparation can also serve as supplement in case of protein mal-nourishments. It can also be applied as a valuable substitute for conventional cow milk and other known milk sources. The tiger nut varieties' milk has comparable values, so it does not really matter which of the varieties is consumed. However, the mixture of the varieties of the milk preparations exhibited synergistic effect in some cases as it combined some beneficial

amino acid that either of the two varieties lacks.

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