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Research Article

Determination of Tryptophan Content in Hausa Koko (Spicy Millet Porridge): A Ghanaian Beverage

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Abstract

Hausa koko is a soft spicy millet porridge commonly consumed in Ghana. The beverage has been postulated to cause drowsiness which may be attributed to the tryptophan available in the pearl millet used in the preparation. Knowledge of this effect may significantly impact consumers' lives and as such, a scientific evidence to support this claim is necessary. This study aimed at estimating the quantity of tryptophan in a given amount of *Hausa koko* and to relate the amount present to the ability of *Hausa koko* to induce drowsiness. A survey was conducted on the possibility of *Hausa koko* causing drowsiness. The presence of tryptophan in both pearl millet powder and *Hausa koko* was established using the Hopkin Cole's reaction. The protein content of the pearl millet powder and *Hausa Koko* were determined using the Kjeldahl's digestion and distillation apparatuses. The quantity of tryptophan was determined from the protein content and the amount of tryptophan in *Hausa koko* estimated with reference to literature values. A protein content of 7.61%±0.07 and 2.2%±0.02 were obtained for pearl millet and *Hausa koko* respectively. A 100g of *Hausa koko* was found to contain 0.27g of tryptophan and by extrapolation, the amount of tryptophan in 500ml of *Hausa koko* was 1.73g. The estimated tryptophan in *Hausa koko* is within a range (1g–15g) that induces drowsiness.

Keywords: Drowsiness, Pearl millet, Hausa koko, Tryptophan.

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1. Introduction

Soft porridges are popularly consumed street-vended local foods in some African countries. They are usually prepared from cereals (particularly millet and maize). In Ghana, an example of such porridges is spicy millet porridge, popularly known as "*Hausa koko*", a porridge whose production and consumption originated from the northern part of Ghana where pearl millet (*Pennisetum glaucum*) is mostly grown [1]. Pearl millet ranks as the world's fourth, the most tropical cereal consumed. The nutritional content makes it a target source of protein and carbohydrate for energy provision [2]. In comparison with other cereals, it is known to have low glycemic index but high protein content in addition to high levels of zinc, iron and antioxidants [3]. Tryptophan is one of the essential amino acids that makes up the protein content of pearl millet. Compared to other millet species, the relatively high amount of tryptophan in pearl millet proteins is of significant biochemical value [4]. Tryptophan is a large neutral essential amino acid that needs to be supplied via dietary intake or nutritional supplementation [5]. The syntheses of the neurotransmitter serotonin (5-hydroxytryptamine) and the pineal hormone melatonin (5-methoxy-N-acetylcholine) begin with tryptophan as a precursor [5-7]. Serotonin and melatonin are naturally produced biomolecules involved in the normal production of sleep. Hence, tryptophan-containing diet causes a likely increase in the level of serotonin and subsequently

melatonin which is able to influence both the quality and quantity of sleep. In addition, tryptophan is involved in the synthesis of other biomolecules including kynurenine and niacin [7].

Unpublished reports of observations of drowsiness experienced by individuals who consume *Hausa koko* have been made over the years. However, little or no scientific evidence exists to support that *Hausa koko* indeed elicits such effect and the identification of the chemical entity implicated in causing such an effect is still in suspense. It has however been postulated that the drowsiness effect associated with *Hausa koko* could be from the presence of tryptophan in the pearl millet used but this has not been scientifically proven.

The prevalent consumption of *Hausa koko* in Ghana calls for the need to establish the possible beneficial and adverse impacts of the beverage on certain groups of individuals such as students, business owners, commercial and private drivers. These groups form an integral part of the productive population in the country hence, their socio-economic influence cannot be over-emphasized. The drowsiness effect of *Hausa koko*, may however be used therapeutically in the management of certain conditions including insomnia, depression and anxiety.

This study investigates the presence of tryptophan in pearl millet and to estimate the protein content and subsequent quantification of tryptophan in pearl millet and in *Hausa koko* based on the assumption that all proteins in *Hausa koko* is solely from the pearl millet. The data obtained will be extrapolated to determine how a volume of *Hausa koko* consumed can induce drowsiness.

2. Materials and methods

2.1 Samples and reagents

Pearl millet seeds were obtained from Ayeduase market (Kumasi, Ghana), stored in a bag, kept away from light and moisture andan amount processed separately into powder with a blender and stored in different plastic bags. Millet porridges (*Hausa koko*) were obtained from the streets of Ayeduase, Kotei and the Campus of KNUST and stored away from light and moisture. Pure tryptophan powder was supplied by the Department of Biochemistry, KNUST and appropriately stored. All chemicals, including Boric acid, Sodium Hydroxide, Hydrochloric acid (HCl) and mixed indicator (methylene blue and phenol red) were of analar grades. The reagents, concentrated sulphuric acid (VWR Chemicals) and Kjedahl catalyst (1g of Na_2SO_4 and 0.1g $CuSO_4$) were of analar grade.

2.2 Survey

A purposive survey was conducted by distributing questionnaires to 215 respondents via emails, social media network, phones as well as face-to-face communication. Table 1 shows the content of the questionnaire and processed percentage responses.

2.3 Qualitative test for tryptophan (hopkin-cole's test - glyoxylic acid reaction)

The test was conducted on the pure tryptophan (control), pearl millet powder and *Hausa Koko*. For each sample in various test tubes, 3ml of glacial acetic acid was added and mixed thoroughly. The tubes were inclined and 5ml H_2SO_4 (concentrated) was gently added from the sides of the tubes. The mixtures were gently warmed in a water-bath for at least 2 minutes and the test-tubes allowed to stand.

2.4 Determination of protein content

The protein content of the pearl millet powder and *Hausa Koko* were determined using the Kjeldahl's digestion (Labconco Corporation) and distillation apparatuses (Kjeltec System Distillation Unit, Tecator). Amounts of pearl millet and *Hausa Koko* samples weighing between 0.2-0.4g were weighed and transferred into individual digestion tubes. 10ml H_2SO_4(concentrated) was added to each sample in the digestion tubes and sufficient amount of Kjeldahl's catalyst[8] were added respectively to the tubes. The tubes were placed in a Kjeldahl digester for 3hours at 375°C[9] and samples allowed to digest completely till observed to be clear. The tubes were removed from the digestion apparatus after digestion and the solutions transferred into 50ml centrifuge tubes. The solutions were made up to 50ml using distilled water and 10ml of each sample solution pipetted into kjeldahl distillation tubes to which 10ml of 40% NaOH were added to neutralize the solution. 10ml of 4% boric acid solution was pipetted into 250ml conical flasks and 4-5 drops of mixed indicator were added. The neutralized solutions in the distillation tubes were put into the kjeldahl distillation apparatus and the conical flask containing the boric acid and mixed indicator were positioned at the receiving ends of condenser of the apparatus. The sample solutions were distilled off into the boric acid solution. The solution obtained in the conical flask after each distillation was titrated with 0.017605N hydrochloric acid to a pink end point. The titrations were carried in triplicates for each sample solution. The percentage nitrogen content (%Nitrogen) and subsequently the total protein content of samples were estimated using equations 1 and 2.

$$\%\ N = \frac{D * N(HCl) * 14.01 * titre\ Value}{1000 * Weight\ of\ sample} * 100 \quad \text{..... Equation 1}$$

Where *N (HCl)* is the Normality of Hydrochloric acid
D is the Dilution factor

$$\text{Total protein content} = \%\ N * 6.25 \quad \text{..... Equation 2}$$

Where 6.25 is the conversion factor. [10]

3. Results

3.1 Survey results

Table 1: Questionnaire and percentage (%) responses on the possible drowsiness caused by *Hausa koko*.

A. Personal Data					
Age (years)	*%*	*Weight (kg)*	*%*	*Education level*	*%*
15-20	31.2	20-39	0.9	Primary	0.5
21-30	60.9	40-59	34.4	Secondary	2.8
31-40	5.1	60-75	46.0	Tertiary	95.4
Above 40	2.8	Above 75	18.6	Other	0.5
B. Consumption of *Hausa Koko*					
How often do you take in Hausa koko?	*%*	*What time of the day do you usually take it?*	*%*	*Quantify the amount that you take in*	*%*
Always	3.8	Morning	83.9	250ml (Ghc 0.5)	14.0
Often	42.5	Afternoon	1.9	500ml (Ghc 1.0)	76.3
Scarcely	52.8	Evening	3.3	750ml (Ghc 1.5)	9.8
Never	0.9	Any time of the day	10.9		
B. Effects of *Hausa Koko*					
Q1: Does Hausa Koko ever make you drowsy?	*%*	*Q2: After how long do you feel sleepy when you take in Hausa koko?*	*%*	*Q3: In your own opinion, is Hausa Koko able to induce sleep?*	*%*
Yes	58.0	Within an hour	48.1	Yes	62.0
No	21.0	Over an hour	20.6	No	12.0
Can't tell	21.0	Never	13.1	Can't tell	26.0
		I don't really know	18.2		

3.2 Qualitative test (Hopkin Cole's reaction)

Figure 1 shows the results of the Hopkin Cole's test where a violet ring is observed between the two interphases, an indication that tryptophan is present.

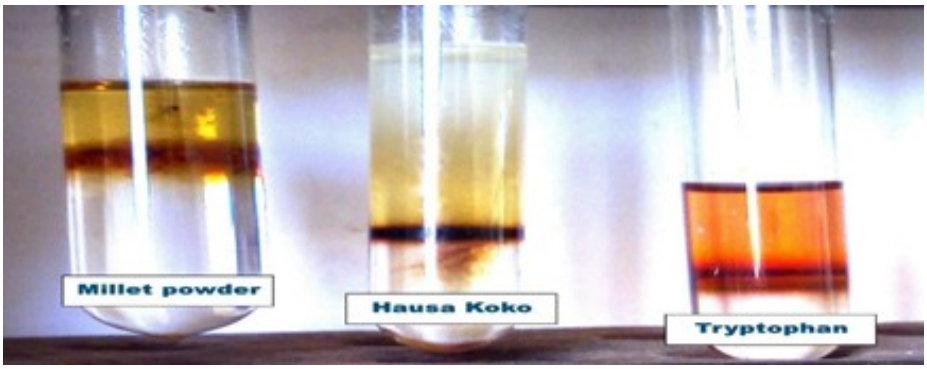

Figure 1: Test tubes showing violet rings for presence of tryptophan

3.3 Protein content determination

Using the Kjedahl's apparatus, the condensates were used for the titration and subsequent determination of %N and protein content.

Table 2: Determination of %N and protein content

Sample	Weight	Titre value(ml)	%N	Total protein (%)
Powdered pearl millet	0.22	2.15	1.22	7.61
Hausa Koko	0.21	0.60	0.33	2.20

Sample calculation of % N and total protein content for powdered pearl millet

D (50/10) = 5

Weight of sample = 0.22g

$$\% N = \frac{5 * 0.017605 * 14.01 * 2.15}{1000 * 0.22} * 100$$

$$= 1.22\%$$

Total protein content = % N * 6.25

= 1.22 * 6.25

= 7.61%

3.4 Estimation of tryptophan content

A 100g pearl millet contains an average of 11g protein with approximately 1.45g of tryptophan [11].

$$\text{Therefore: 7.61g of protein} = \frac{7.61}{11}\ x\ 1.45g$$
$$= 1.00\text{g tryptophan}$$

For the *Hausa koko*, the assumption is that only the pearl millet contributes to the total protein content and for that matter all the tryptophan.

From the results, a quantity of 100g of *Hausa koko* contains 2.2g of protein

$$\text{Therefore, 2.2g of protein} = \frac{2.2}{11}\ x\ 1.45g$$
$$= 0.29\text{g tryptophan}$$

From the survey, the average individual takes in approximately 500ml of *Hausa Koko*. With an average density of 1.19g/ml of sampled *Hausa koko*

$$\text{500ml of } \textit{Hausa Koko} = \frac{500}{100}\ x\ 119.12g$$
$$= 595.6\text{g}$$
$$\text{595.6g of } \textit{Hausa Koko} = \frac{595.6}{100}\ x\ 0.29g$$
$$= 1.73\text{g}$$

Hence a volume of 500ml *Hausa Koko* contains approximately 1.73g of tryptophan.

4. Discussion

4.1 Survey

Table 1 shows the results of the survey with the majority of the respondents aged between 20-30, weighing 60-70kg and having attained tertiary education. This group further represents an elite and working class with an understanding of the issues raised with the patronage of *Hausa koko.* The survey in addition indicated that consumers largely take the beverage in the morning with an average volume of 500ml costing Gh₵ 1.0 often times but may scarcely do so. Close to 60% of respondents attest to being drowsy within an hour or more when they take in *Hausa koko,* and in their opinion, the beverage is responsible for the observed effect.

4.2 Hopkin Cole's reaction

The Hopkin Cole's reaction is specific for the amino acid tryptophan and the positive result as obtained is an indication that tryptophan is present in all three samples (Figure 1). Furthermore, the reaction is between the indole ring of tryptophan and the glyoxylic acid and hence any other compound or substance which has the indole ring is likely to give a positive result. However, to the best of our knowledge, in *Hausa Koko*, no other ingredient is known to contain the indole ring except for the tryptophan in the pearl millet used for the preparation of the beverage, confirming the presence of tryptophan.

4.3 Protein content

The total amount of protein determined for the pearl millet and the *Hausa koko* were 7.61%±0.07 and 2.2%±0.02 respectively. Pearl millet has been found to contain up to about 13% protein, but large differences in value exist among various genotypes and a range between 6% and 21% has been reported [14]. In comparison with the 11% quoted by Obilana [11], the value obtained in this experiment is lower. Nevertheless, the value obtained falls within the range of expected protein content for pearl millets.

With the protein content of *Hausa Koko*, the value was recorded for the samples using their average density since the protein content is directly proportional to the density but may vary from vender to vender. The vendors however prepare the *Hausa koko* with similar consistency with respect to thickness irrespective of sale site. This indicates that for most of the *Hausa koko* sold on the market; a protein content of the average 2.2%±0.02 estimated in this work may be obtained. To the best of our knowledge, this is the first report of the protein content of *Hausa koko.*

4.4 The amount of tryptophan

It has also been established that the availability of tryptophan in the brain directly correlates with the ability of the body to produce serotonin and melatonin [7]. Reports indicate that the amount of tryptophan to induce sleep varies from individual to individual however a dose between 1g to 15g is effective in eliciting drowsiness[15]. From the results, it was observed that the average person consumes about 500ml of *Hausa Koko* which approximately weighs 595.61g and corresponds to1.73g tryptophan. This amount falls within the quantity of tryptophan required to induce drowsiness and will justify why people feel drowsy when they consume *Hausa Koko*.

There is a transporter in the brain known to transport both tryptophan and the other branched chain amino acids (BCAA). Their relative abundance affects which one will be transported. Hence for tryptophan to get into the brain easily, the tryptophan concentration: other BCAA ratio must be very high so that there may be less competition with tryptophan [7]. *Hausa Koko* (60% carbohydrate) is usually taken together with other carbohydrate containing foods like *koose*, sugar and bread which induces the secretion of high levels of insulin in the body. Insulin being an anabolic hormone causes the uptake of the BCAA into the muscles and tissues resulting in a high tryptophan to BCAA ratio [7]. Such availability of tryptophan favors its uptake into the brain and hence most of the approximate 1.73g estimated will be available for uptake into the brain.

Furthermore, tryptophan is lipophilic and is appreciably bound to and transported by albumin in plasma (about 75 -85% bound to albumin) [7]. Insulin however makes albumin less saturated in terms of its lipid carrying capacity [16] and increases the albumin's binding affinity for tryptophan leading to a decrease in the unbound tryptophan levels in the blood. However, this decrease is offset by the increase in the amount of bound tryptophan. Albumin is able to bind to more tryptophan at a time but it does so with a low affinity. Since albumin's attraction is significantly weaker than that of the passage of macromolecules in various brain vessels, up to 70% of the bound tryptophan is as available for brain usage as "free" tryptophan would be [16].

The foregoing discussion therefore supports the assertion that *Hausa koko* exerts a drowsiness effect on its consumer due to the presence of adequate amount of tryptophan in the beverage. However the drowsiness effect of *Hausa koko*, if properly harnessed, could be therapeutically effectively in the management of other conditions including insomnia, depression and anxiety. Patients with temporary sleep disorders may also benefit from the beverage (as health food) that will exhibit less untoward side effects compared to allopathic medication.

5. Conclusion

The purposive survey has revealed that approximately 58% of respondents attested to the fact that *Hausa Koko* has capacity to induce drowsiness effect. The Hopson Cole's reaction established the presence of tryptophan in pearl millet and *Hausa Koko.* The Kjeldahl's digestion and distillation apparatuses were employed in quantifying the protein content of *Hausa Koko* with an estimated value of 2.2 % $^{w}/_{w}$. By extrapolation, 500ml (equivalent to 595.6g) of *Hausa Koko* was found to contain 1.73g of tryptophan, an amount adequate to induce drowsiness in patrons of the beverage. This study has therefore determined that indeed *Hausa koko* induces drowsiness due to the presence of tryptophan in the beverage.

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