

Comparative Study of Two Millet-Based Foods Enriched With Cashew Kernel and Tiger Nut

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Abstract: In a context of food insecurity, animal proteins, although considered a reference in terms of nutritional quality, remain inaccessible to many households, hence the use of vegetable proteins which represent an opportunity to meet needs. However, the latter are often less well balanced in essential amino acids than animal sources, which requires complementarity between sources. In order to promote local products of plant origin, a comparative study of two fortified foods was carried out to determine their vitamin A and E content and their amino acid profile. One binary obtained from millet flour enriched with 14.30% cashew kernels and the other ternary obtained from millet flour enriched with 5% cashew kernels and 11.80% tiger nut. The results of the vitamin A and E contents in the binary mixture were respectively 57.5 ± 0.5 mg/100g and 14.95 ± 0.106 mg/100g against respectively 71 ± 2.30 mg/100g and 19.5 ± 0.1 mg/100g in the ternary mixture. The vitamin contents of the ternary mixture were significantly higher ($P \leq 0.05$) than those of the binary mixture. The amino acid profile indicates the presence of all eight essential amino acids in both samples. However, lysine and isoleucine with respectively contents of 3.61 ± 0.03 mg/g and 4.2 ± 0.004 mg/g, in the binary mixture against 4.02 ± 0.036 mg/g and 3.74 ± 0.05 mg/g, in the ternary mixture was the most representative. In sum, fortification improved the nutritional value of composite flours. This could therefore be an alternative to the problem of malnutrition and a solution in households where access to animal products remains a problem.

Keywords: Millet, Tiger Nut, Cashew Kernel, Amino Acids

1. Introduction

The Ivorian population is a victim of the double burden of malnutrition which is characterized by overnutrition (obesity, overweight) and undernutrition (stunting, acute malnutrition, underweight) [1]. Malnutrition usually occurs at the age of 6 months when breast milk alone is not enough to meet the energy needs of the child [2]. It is therefore recommended the incorporation of complementary food. Complementary foods or weaning foods are used as a complement to breast milk when the child reaches his 6th month [3]. Complementary foods are the first foods an infant consumes when breast milk becomes insufficient to meet its needs. This food must be nutritionally rich because it is designed to meet the nutritional needs of the child [2] like commercial flours.

Imported commercial flours are solutions to meet the energy needs of infants, but their inaccessibility by most households due to their high cost makes them a limiting factor. An alternative to this situation is the use of locally accessible raw materials to cover the infant's needs [2].

In Côte d'Ivoire, complementary foods are generally based on cereals and tubers [4]. Millet (*Pennisetum glaucum*) is one of the most widely grown cereals in southern countries. This cereal has a relatively higher nutritional value than rice, wheat and maize and is often used to make complementary food for children [5]. But like most cereal products and tubers, millet does not have sufficient protein and micronutrient levels to meet the nutritional needs of children

[6]. An alternative in the fight against malnutrition is therefore the enrichment of these local products [7]. Pulses have often been used for the fortification of complementary foods, among them the cashew kernel. The cashew kernel is an important source of nutrients. This legume, because of its richness in nutrients (micro and macronutrients), could be used to fortify millet-based flours [8]. It has good protein content [9]. Cashew nuts have good nutritional value due to their protein (20-24g/100g), carbohydrate (23-25g/100g), lipid (40-57g/100g) [10-12] and sugar content. minerals [13]. However, the content of bioactive compounds could also be reinforced by other local products such as tiger nut. The tiger nut is presented as an alternative in the fortification of complementary food because of its richness in fibers, polyphenols, antioxidants, vitamins, bioactive compounds [14, 15] and essential amino acids [16].

In this work, it was a question of determining the nutritional value of two compound foods made from local products in order to offer them to households for the fight against child malnutrition.

2. Materials and Methods

2.1. Materials

Three local raw materials were used. These are cashew kernel (*Anacardium occidentale* L.), tiger nuts (*Cyperus esculentus*) and millet (*Pennisetum glaucum*). The millet and tiger nut were obtained from the market in the city of Yamoussoukro (6°53'04.7" North and 5°13'54.9" West) in the center of Côte d'Ivoire. The cashew kernels were supplied by the school factory of the National Polytechnic Institute of Yamoussoukro (Côte d'Ivoire).

2.2. Methods

2.2.1. Production of Millet Flour

A quantity of 500 g of millet bought on the market is sorted and washed three times in a row. The millet grains were dried in an electric dryer with racks at 70°C for 17 h. A Philips Laboratory Blender (model HR2811) with a mesh diameter of 60 mm was used to sieve the millet grains. The sieved millet was stored in hermetically sealed plastic bags and left at room temperature.

2.2.2. Production of Downgraded Cashew Kernel Flour

The production of downgraded cashew kernel flour was inspired by the method described by Sze-Tao K. W. and Sathe S. K. [17]. The downgraded cashew kernels obtained are crushed using a semi-artisanal grinder (YIBU TYPE 30 China). The crushed almonds were passed through an electric rack dryer at 70°C. for 17 hours. The dried almonds are ground using a 200 micron sieve grinder. The resulting almond flour is stored in plastic jars at room temperature.

2.2.3. Production of Tigernut Flour

The tiger nut seeds purchased at the large Yamoussoukro market were sorted to remove impurities and then washed several times with clean water. The beans were dried in an

electric rack dryer and roasted at 100°C. for 45 min in an open roaster. The grains are then removed and placed in a stainless steel tray for cooling and then crushed in a hammer mill. The tiger nut flour obtained is stored in plastic bags for conservation while awaiting analysis.

2.2.4. Formulation of Mixed Flours

Two qualities of mixture were elaborated: a binary mixture and a ternary mixture. These proportions were selected following a mixing plan which resulted in these levels via the expert design software.

Table 1. Binary mixture of millet and cashew kernel flour.

local products	millet	downgraded cashew kernel
quantity (%)	85.70	14.30

Table 2. Ternary mixture of millet flour, cashew kernel flour and tiger nut.

local products	millet	downgraded cashew kernel	tiger nut
quantity (%)	83.20	5	11.80

2.2.5. Physico-chemical Analysis

The analysis s are carried out in triplicate in order to average them for the determination of the parameter considered.

Proteins

The Kjeldal method was used to determine the crude protein content. The percentage of nitrogen (%N) was used for the calculation of the crude protein content (%P) from the equation below [18].

$$\% P = 6.25\% N$$

The protein assay was done by HPLC (High Performance Liquid Chromatography) by undergoing acid hydrolysis of their proteins.

Amino acids

The method inspired by Moore S. and William S. [19] was used for the preparation of hydrolysate samples. A volume of 200 mg of each sample and a volume of 5 ml of 6 N HCL were withdrawn gradually and introduced into tightly closed hydrolysis tubes. The whole is incubated at 110°C. 24 hours later the tubes are removed from the incubator and filtered. Dry evaporation at 140°C. for 1 hour was carried out on 200 mL of the filtrate. The hydrolysates were dried and then diluted with 1 mL of 0.12N citrate buffer, pH 2.2. In a cation separation column at 130°C, a volume of 150 µl of aliquot sample was injected. A high performance reactor with a flow rate of 0.7 mL/min is used to receive a solution of ninhydrin and a buffer eluting concomitantly. In order to accelerate the chemical reaction of the amino acids with the ninhydrin solution, the mixture consisting of the buffer solution and the ninhydrin was heated in the reactor at 130°C for 2 minutes. A double channel spectrophotometer made it possible to read the wavelengths respectively at 570 nm and at 440 nm of the mixture. The amino acid profile was obtained from the surfaces of the standards obtained.

Dosage of fat-soluble vitamins (A and E)

The extraction of fat-soluble vitamins (A and E) was carried out according to the method described by Jedlicka A. and

Klimes J. [20]. To 1.0 g of the sample was added 10 mL of a 10% KOH solution in methanol-water (1:1, v/v). To avoid the oxidation process during saponification, 0.025 g of ascorbic acid was added. The mixture is then refluxed in a water bath at 70°C. for 30 min. the mixture is then cooled and extracted with 3 x 5mL of hexane. The hexane phases were combined and dried over anhydrous sodium sulphate then evaporated to dryness. The residue obtained (approximately 0.3 g) is taken up in methanol (10 mL) for analysis. The evaluation of fat-soluble vitamin content was made by HPLC coupled to a fluorimetric detector. The analysis is carried out in isocratic mode on a Hypersil ODS RP18 column (stationary phase), 5 µm particle diameter and 4.6 mm diameter. The mobile phase is an Acetonitrile/methanol mixture (80:20, v/v) with a flow rate of 1 mL/min. The standards were prepared by dilution series (1/10th then 1/2):

α -tocopherol (E): 3.4µg/100mL;

Retinol (A): 11.3 µg/100 mL;

All calculations are made from the 100% witness.

Fluorimetric detection: vit A (455 nm), vit E (295 nm).

Polyphenols

The modified Singleton and Rossi method [21] was used for the determination of polyphenols. An extract with a volume of 300 µl was prepared. To this extract was added respectively 4.2 mL of distilled water, 0.75 mL of Folin Ciocalteu's reagent and then 0.75 mL of 20% (W/V) sodium carbonate. Each time the reagent is added, the solution is stirred and then incubated for 30 minutes. Different concentrations (0 to 1000 micrograms) of gallic acid were

used to produce the calibration curve.

Flavonoids

The flavonoid assay protocol is based on that described by Zhishen and *al.* and Kim and *al.*, [22, 23] modified. In a glass tube containing 120 µl of 5% NaNO₂ was added 400 µl of extract or standard or distilled water (control). A quantity of 120 µl of 10% AlCl₃ was added 5 min later. The whole was stirred and 6 min later 800 µl of 1M NaOH were introduced into the mixture. The optical density was read at 510 nm against the control. A calibration curve was drawn from the methanolic solution of quercetin.

Carotenoids

A quantity of 2 g of each sample is introduced into a tube. Two times 2 mL of hexane is added in the presence of echinenone at a concentration of 0.6 pmol/µl. The solution was stirred and then centrifuged at 3000 rpm min⁻¹ for 5 min at - 5°C. the hexane phases are recovered and passed over a nitrogen stream for evaporation. The residue obtained is added to 800 µl of acetonitrile in order to obtain a solution containing 15 pmol/20 µl of the internal standard. A volume of 20 µl was injected [24].

2.2.6. Statistical Analysis of Data

Statistical analysis were performed with Statistica 7.1 software. The results are expressed as the mean \pm standard deviation of the triplicate measurement. An analysis of variance (ANOVA) was carried out and the significance of the differences between the flour samples is determined at the risk of error of 5% according to the student test.

3. Results

Table 3. Protein contents in the basic matrices.

parameters	downgraded cashew kernel	tiger nut	millet
protein content (%)	18.38 \pm 0,08	6.13 \pm 0,031	7.88 \pm 0,31

Table 4. Amino acid content in the binary mixture (mg/g).

downgraded cashew kernel (%)	millet (%)	lysine	L-méthionine	Histidine	isoleucine	leucine
14.3	85.7	3.61 \pm 0.03	0.08 \pm 0.002	0.34 \pm 0.01	4.20 \pm 0.004	1.91 \pm 0.017

Table 4. Continued.

downgraded cashew kernel (%)	valine	phénylalanine	thréonine	tryptophane	tyrosine	arginine
14.3	0.88 \pm 0.041	2.04 \pm 0.01	1.23 \pm 0.013	0	1.46 \pm 0.001	1.80 \pm 0.002

Table 5. Amino acid content in the ternary mixture (mg/g).

downgraded cashew kernel (%)	tiger nut (%)	millet (%)	lysine	L-méthionine	histidine	isoleucine	leucine
5	11.80	83.20	4.02 \pm 0.036	0.05 \pm 0.03	0.37 \pm 0.02	3.74 \pm 0.05	2.04 \pm 0.01
Standards AFSSA* (2007)			1,20	0,33	0,43	0,58	1,25

Table 5. Continued.

downgraded cashew kernel (%)	valine	Phénylalanine	Thréonine	tryptophane	tyrosine	arginine
5	0.68 \pm 0.02	2.16 \pm 0.01	1.07 \pm 0.034	0	1.53 \pm 0.03	1.87 \pm 0.034
Standards AFSSA* (2007)	0,78	0,68	0,68	-	0,50	-

* AFSSA: Agence Française de Sécurité Sanitaire des Aliments (French Food Safety Agency).

Table 6. Contents of bioactive compounds and vitamins A and E in the binary mixture (MB).

Downgraded cashew kernel (%)	Millet (%)	Polyphénol (mg/100g)	Flavonoïd (mg/100g)	Caroténoid (mg/100g)	vitamin A (mg/100g)	vitamin E (mg/100g)
14.3	85.7	104.5±1.73	16.25±0.026	1.55±0.132	57.5±0.5	14.95±0.106

Table 7. Contents of bioactive compounds and vitamin A and E in the ternary mixture (MT).

Downgraded cashew kernel (%)	tiger nut (%)	Millet (%)	Polyphénol (mg/100g)	Flavonoid (mg/100g)	Caroténoid (mg/100g)	Vitamin A (mg/100g)	Vitamin E (mg/100g)
5	11.80	83.20	102±2.64	33.12±0.09	1.5±0.05	71±2.30	19.5±0.1

4. Discussion

Two samples were used for the study. The sample made from millet (85.70%) enriched with downgraded cashew almond (14.30%) is a binary mixture called MB and the sample made from millet (83.20%) enriched with cashew almond (5%) and au shot (11.80%) is a ternary mixture called MT. Three local raw materials were used for the formulation of two qualities of supplement food. Protein content of basic samples (Table 3) varied from $6.13 \pm 0.031\%$ to $18.38 \pm 0.08\%$. The largest content was observed in the cashew almond, 18.38%. High protein contents had been indicated in cashew almond [25]. The lowest protein content has been observed in the slut, this could be explained by drying because a well -led drying would decrease the protein content of the stack [26]. The protein content of millet is significantly equal to that of wheat and corn [27]. The millet even if it presents a higher protein content in this study that the tiger nut is not however considered a reliable source of protein because the majority of millet proteins would be of poor quality. It is generally made up of prolamin which is a protein of poor quality [27]. However, millet despite this remains one of the most appreciated cereals for these nutritional benefits among cereals. Of all the cereals produced in Sahelian Africa, millet and rice have the best amino acid profile. The nutritional value of a protein is related to its ability to meet the body's needs for nitrogen and essential amino acids [28]. The results indicate high concentrations of amino acids in binary (table 4) and ternary (table 5) mixtures. These high concentrations of amino acids would therefore be due to the combined effect of millet and cashew kernels.

The results of the amino acid contents of the binary mixtures (Table 4) indicate the presence of several amino acids including the essential amino acids. It should be noted high concentrations of certain amino acids such as lysine ($3.61 \pm 0.03\text{mg/g}$), isoleucine ($4.20 \pm 0.004 \text{ mg/g}$), phenylalanine ($2.04 \pm 0.01 \text{ mg/g}$) compared to other amino acids. These amino acids have important physiological roles in the development of children. This could be due to the richness of the cashew kernel in amino acids, the high rate of which in the mixture certainly contributed to improving the amino acid profile [29]. In the ternary mixture (Table 5), although the cashew kernel content drops (it went from 14.30% in the binary mixture to 5% in the ternary mixture), an improvement is observed in the contents of lysine, histidine, leucine and phenylalanine compared to the contents

of these amino acids in the binary mixture. This could be due to the presence of tiger nut in the ternary mixture and the effect of roasting which would improve the amino acid content as mentioned by Ijarotimi O. S. and *al.*, [29].

The amino acid values observed in the binary and ternary mixtures are all higher than the standards indicated by the French Food Safety Agency (AFSSA) for infant foods (Table 5) with the exception of L-methionine and histidine which exhibit 0.33 and 0.43 mg/g respectively. The high amino acid contents in the binary and ternary mixtures show the effect of the combination on improving amino acid contents.

Reducing the cashew kernel content in the mix without negatively impacting the amino acid profile of the mix is an asset because although the cashew kernel is a local product available in high quantities in Côte d'Ivoire, it is however inaccessible because of its price which is not within the reach of all budgets. It was therefore important in this work to offer a final composite product of good nutritional quality while minimizing the cashew kernel content. It should be noted that no cereal has sufficient levels of amino acids to meet the growth needs of infants and young children. However, of all the cereals grown in sub-Saharan Africa, millet presents with rice the best amino acid profile [28], which justifies the choice of millet as the staple food for the formulation of complementary food in this study.

The contents of bioactive compounds and vitamin A and E of the binary mixture are presented in Table 6. A high content of polyphenols, flavonoids, vitamins A and E is observed, which are respectively $104.5 \pm 1.73\text{mg}/100\text{g}$, $16.25 \pm 0.026\text{mg}/100\text{g}$, $57.5 \pm 0.5\text{mg}/100\text{g}$ and $14.95 \pm 0.106\text{mg}/100\text{g}$. The contents of polyphenols, flavonoids, vitamin A and vitamin E in the ternary mixture (Table 7) are respectively $102 \pm 2.64 \text{ mg}/100\text{g}$, $33.12 \pm 0.09 \text{ mg}/100\text{g}$, $71 \pm 2, 30\text{mg}/100\text{g}$, $19.5 \pm 0.1\text{mg}/100\text{g}$. There is an increase in the concentrations of flavonoids and vitamin A and E from the binary mixture to the ternary mixture, although the cashew kernel contents drop. The improvement in levels would be due to the high levels of bioactive compounds and vitamins A and E in tiger nut [14, 15].

The decrease in the cashew kernel content and the incorporation of tiger nut allowed a slight improvement in the content of polyphenols, flavonoids and vitamin A and E. Table 6 indicates the vitamin A and E contents of the binary mixture. These contents are markedly improved in the ternary mixture with the presence of the tiger nut. It is well documented that vitamin E is mostly of plant origin [30]. Tiger nuts would contain high levels of vitamins A and E

[14, 15]. This remains a considerable asset because vitamin and mineral deficiency in some countries would result in the loss of 5% of their gross national product and this, in loss of human life, disability and reduced productivity [31]. The results obtained (Table 7) following the incorporation of the tiger nut in the ternary mixture would be due to the richness of this tuber in bioactive compounds and vitamin E. The vitamin E content increased from 14.95 ± 0.106 mg/100g in the binary mixture at 19.5 ± 0.1 mg/100g in the ternary mixture. This increase in vitamin E content helps to improve the nutritional value of the mixture because vitamin E as an antioxidant is known for its ability to inhibit lipid peroxidations and fight against free radicals and non-radical elements produced during the formation of free radicals [30].

The fight against child malnutrition involves not only the use of local products in the development of infant flour [2] but also and above all the use of compound foods made from legumes. Given that legumes contribute significantly to the nutritional quality of infant foods, it is more than essential to use them in the establishment of complementary foods, however they are very little used for this purpose. This practice will have a double advantage: firstly, to improve the nutritional quality of food, then it will contribute to the promotion of tiger nut in Côte d'Ivoire. A second alternative is to encourage households to use compound flours as a complementary food because this has the advantage of improving the quality of the final product. A study by Kayode A. P. P. and *al.*, [32] indicated that only 7% of households use compound flours as complementary foods. While one study had indicated that the combination of two or more plant-based food materials would improve the basic nutritional composition and quality of the formulated product [29]. The results obtained in this work are significantly lower ($P \leq 0.05$) than those obtained by Ijarotimi and *al.*, [29] in the formulation of composite flour based on maize enriched with cashew kernel. This difference can be explained by the technological methods used, i.e. the fermentation, germination and roasting of the cashew kernel. It is recognized that technological methods such as fermentation and sprouting would increase the bioavailability and content of nutrients in food products [29]. The use of flours made from local products would therefore be a sustainable local solution to the fight against child malnutrition.

5. Conclusion

The objective of this study was to propose composite formulations based on local products which will have good levels of amino acids, bioactive products and vitamin A and E. Two qualities of food have been developed. A binary mixture obtained from millet enriched with downgraded cashew kernels and a ternary mixture based on millet enriched with cashew kernels and tigernuts. The results indicated the presence of essential amino acids in both types of formulations, however the ternary mixture was better reinforced in polyphenols, flavonoids and vitamins A and E due to the incorporation of tiger nut. This makes it possible to promote local products and encourage households to use composite foods for the formulation of complementary foods which would be an asset against the

malnutrition observed in infants.

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