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# Effect of Different Packaging Materials on Caffeine Content in Fresh Nuts of *Cola nitida* from Côte d'Ivoire

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**ABSTRACT**

Kola nuts (*Cola nitida*), which is a great source of caffeine, faces a real problem of conservation for the actors of the sector. The objective of this study is the validation of a quick, sensitive and reliable method of quantification of caffeine of kola nuts by UV-Vis spectrophotometry and evaluation of the influence of three types of packaging (basket, trays in PS and bag triple bagging) during conservation. Kola nuts were collected in the region of San Pedro (South-West Ivory Coast), then transferred to the laboratory for experiments with the prior validation of a caffeine extraction method. Validation parameters concerned: Linearity Limit (LL), limit of detection (LOD), limit of quantification (LOQ) and Coefficient of Variation (CV). The standard concentration range of pure caffeine was: 0.003 to 0.01 g/l. During storage, water contents of kola nuts were determined by drying in an oven at 105 ºC for 24 hours and validated method was used for caffeine quantification. The method developed demonstrated very good linearity ($R^2$ = 0.9997) over a concentration range of 0.003 to 0.01 g/l. Fidelity is expressed by coefficient of variation (CV = 0.936 ± 0.589%). The detection limit is $1.705x10^{-4}$ g/l and limit of quantification is $5.683x10^{-4}$ g/l. Otherwise, a good extraction yield was obtained (Θ = 94.749 ± 0.716%). The results obtained after six (6) months of storage of cola showed that only opaque PS trays have preserved physicochemical properties of kola nuts during 6 months.

## INTRODUCTION:

Kola nuts (*C. nitida*) are very popular because of their use in native ceremonies [1, 2]. The current consumption represents, for the most part, the reason for buying kola nuts. Indeed, it represents 17.35% for direct consumption, 14.23% for sacrifices, 14% for dyeing, 13.7% for weddings and 12% for baptisms [3]. It is used as a stimulant, promoting the physical and psychic endurance of manual workers [4, 5] or as ingredients in the formulation of certain pharmaceutical products and energy drinks.

The therapeutic properties reported on kola nut extracts are mainly attributed to bioactive compounds such as caffeine, polyphenols, flavonoids, tannins, saponins and alkaloids [4, 6, 7].

Among these bioactive compounds, caffeine plays a predominant role. Caffeine (1,3,7-trimethyl xanthine) is an alkaloid of the methylxanthine family. It is synthesized by about 60 plant species, the most known of which are the coffee tree, the kola tree and the tea tree [8]. It is one of the most widely consumed natural pharmacologically active substances in the world, mainly as drinks (tea, coffee and energy drinks). According to [9], caffeine stimulates brain activity, increases the speed of reflexes and improves memory. It dilates bronchi blood vessels and accelerates respiratory system movements. It is diuretic and causes digestive secretions. Therefore, caffeine is beneficial to health if it is consumed without excess. In particular, caffeine has the potential to prevent cardiovascular disease, Alzheimer, Parkinson, diabetes, and some cancers such as prostate and breast cancer [9].

Various pharmaceuticals contain synthetic caffeine in combination with other molecules [10]. However, the problem of toxicity usually revealed by the use of synthetic drugs is still relevant and has led to a growing interest in natural sources of bioactive molecules [4, 11]. Thus, kola nuts that are industrially known for their high caffeine content (1.5-3.8%) depending on the variety and nut morphotype, could be used as a natural source of interest of caffeine [4, 12, 13].

Today, Côte d'Ivoire is positioned as one of the leading producers and exporters of kola nuts in the world [4]. However, very little research has focused on the post-harvest component of this product, particularly the conservation and enhancement of fruits [14]. Besides, it is important to determine a precise caffeine content of cola for its best use in industry. This determination would be a valuable information for beverage producers who are interested in other sources of caffeine other than common coffee.

Based on all the above, the objective of this study is to validate a rapid, sensitive and reliable method of quantifying the caffeine of cola nuts by UV-Vis spectrophotometry and to evaluate the influence of three types of packaging during conservation.

## MATERIALS AND METHODS:

### 1. MATERIAL:

**Fresh kola nuts**: mature pulped nuts were collected for 7 days in the San-Pedro area during the month of September 2017. After shelling, 300 kg of untreated pulped nuts were sent to the laboratory in polypropylene bags of 100 kg for their immediate treatment and conditioning.

**Containers for kola nuts:** Three (3) containers were used namely basket lined with leaves of *Thaumatococcus daniellii* (Benn.) Benth., (Container 1), opaque polystyrene [PS] trays, (Container 2) and triple bagging bag (Container 3) containers.

### 2. METHODS:

#### 2.1. Kola nuts treatment and storage:

After cleaning the pulp, sorting, washing, and rinsing, 60 kg of red nuts and 60 kg of white nuts were left drained for 30 minutes before being packaged in the different containers. Three (3) preservation techniques were used for each nut color. After draining, 60 kg of each treated nut color were separated into three batches of 20 kg. Each batch of 20 kg was packaged in a specific container: basket lined with leaves of *Thaumatococcus daniellii* (Benn.) Benth., opaque polystyrene (PS) trays plus food cartons and the triple bagging bag.

#### 2.2. Validation of caffeine quantification method by UV-Vis spectrophotometry

The validation of this analysis method was carried out in accordance with the validation protocol of **[15]** and **[16],** using the following parameters: linearity limit (LL), limit of detection (LOD), limit of quantification (LOQ) and coefficient of variation (CV).

For the determination of linearity, optical densities (OD) of standard dilutions of pure caffeine at concentrations of: 0.003; 0.004; 0.006; 0.007; 0.008; 0.009; 0.01 g/l were read using the UV-Vis Spectrophotometer. The correlation coefficient $R^2$ has been evaluated in order to assess the quality of the calibration curve. The OD was read 10 times on the same solution. The limit of detection (LOD) and the limit of quantification (LOQ) were evaluated from the slope of the calibration curve. Repeatability and precision of the method were

expressed by the coefficient of variation (CV). The coefficients of variation are calculated by the ratio of the standard deviation of the OD read on the average OD. The CV is expressed as a percentage.

### 2.3. Temperature (T) and relative humidity (RH) of the storage magazine

The temperature and relative humidity of the kola nut store were measured twice a day using a miniature thermo-hygrometer (SMART SENSOR AR807).

### 2.4. Moisture content of cola nuts

Humidity levels were determined with 5 g of powdered kola by drying at 105 ° C for 24 hours ± 30 minutes in an oven according to the method described by **[17].**

### 2.5. Extraction of caffeine in the cola sample

From the nut samples (red and white), 200 g of fresh nuts were ground using a blender (Waring Blinder) and served as a laboratory sample for the determination of caffeine content.

Five (5) g of sample were mixed with 150 ml of chloroform. To this mixture was added 0.5 ml of 0.5 mol/l ammonia solution. The resulting mixture was homogenized by magnetic stirring at room temperature (22 ± 2 ° C) for 1 hour. After 1 hour, the supernatant was filtered through pleated filter paper (PRAT DUMAS France, 190 MM, REF: J019106). The operation was performed four times on the same 5 g sample. The filtrates were dried on a rotary evaporator and then recovered with a 0.5 mol/l sulfuric acid solution.

### 2.6. Recovery rate (Ɵ) for caffeine extraction

The operations of exhaustion of the matrix were carried out 4 times on the same sample (5 g) in order to exhaust it completely. The OD was read three (3) times on each extract obtained. In addition, ten (10) exhaustion tests were performed to determine the recovery rate (Ɵ). Recovery rates after extractions and quantification were calculated according to the following formula:

$$\Theta = \frac{C * Vr}{10 * Tc} \qquad (1)$$

*Where Tc: an amount of caffeine extracted in 5 g of sample (in g); C: concentration of caffeine obtained after extraction (in g / L); Vr: recovery volume of caffeine (in l) and Ɵ: recovery rate of caffeine extraction in kola nut at extraction (in %).*

### 2.7. Calculation of the coefficient of variation (CV) on the reading of OD for each extract

Coefficients of variation were calculated for each extract according to the following formula:

**CV = (Standard deviation / Average OD) x 100** **(2)**

### 2.8. Caffeine content of kola nuts

Caffeine content of kola nuts was determined by spectrophotometric analysis. The mixed solution (caffeine-sulfuric acid) was recovered in an Erlenmeyer flask for reading the OD on a spectrophotometer UV-Vis. The absorbance of the solution was measured against a reagent blank (0.5 mol / l sulfuric acid) at 272 nm. The OD has been read 3 times on the same extract. Caffeine concentration determined from calibration curve was used to calculate caffeine extraction efficiency of the kola nuts according to equation (3) taking into account the recovery rate. This caffeine content was determined in grams of caffeine per kilogram of fresh cola.

$$Tr = \frac{C * F * Vr * 10^5}{M * \Theta} \quad (3)$$

*Tr: is caffeine content (in g / kg); C: is concentration of caffeine obtained after extraction (in g / L); F: is dilution factor; Vr: is recuperation volume of caffeine (in L); Θ is recovery rate of extraction of caffeine in kola nut at extraction (in %) and M: is mass (g) of the sample on which the extraction was perform.*

### 2.9. Statistical analyses

After investigating, the collected data have been treated with statistical software SPSS 22.0. Data have been submitted to an analysis of variance (ANOVA) and Tukey post hoc test at a significance level of 5 %.

## RESULTS AND DISCUSSION:

## RESULTS:

### 1- Presentation of the OD measurements of the calibration range

The standard linear calibration curve shows the result obtained **(Figure 1).** We clearly observe a linear relationship between absorbance (OD) and concentrations (C) of standard solutions ($R^2$ = 0.9997).

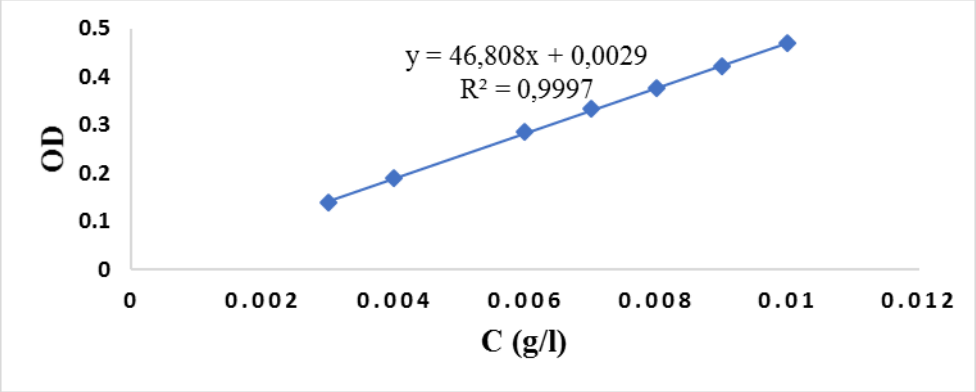


**Figure 1: Calibration curve OD = f (C)**

### 2. Validation of the method

The linearity domain of our method was between 0.003 and 0.01 g/l for caffeine and the linearity expressed by $R^2$ was 0.9997 (Figure 1). The results of the validation tests are shown in Table 1. The limit of detection (LOD) is $1.705x10^{-4}$ g/l, the limit of quantification (LOQ) is $5.683x10^{-4}$ g/l and the variation (CV) of 0.936 ± 0.589%.

**Table 1: Validation parameters**

| Parameters | $R^2$ | LOD (g/l) | LOQ (g/l) | CV (%) |
|---|---|---|---|---|
| Value | 0,9997 | $1,705x10^{-4}$ | $5,683x10^{-4}$ | 0,936±0,589 |

### 3. Presentation of OD measurements and calculation of dilutions of a cola matrix depletion test.

Table 2 presents the results of the OD of a test sample. The first extract is very concentrated, a 1/20 dilution was carried out. Thus, the OD read from the first to the fourth extraction are respectively 0.416 ± 0.002; 0.425 ± 0.001; 0.040 ± 0.001 and 0.009 ± 0.001. We observe that after four successive exhausts of the same sample, the OD values (0.009 ± 0.001) were

practically nil. It was not necessary to perform the fifth extraction. The mean OD values (n = 3) obtained were used to calculate the recovery rates of the different extractions.

**Table 2: OD read extracts from a test sample**

| | **Extract 1** (1/20 dilution) | **Extract 2** (without dilution) | **Extract 3** (without dilution) | **Extract 4** (without dilution) |
|---|---|---|---|---|
| **OD 1** | 0,418 | 0,425 | 0,040 | 0,010 |
| **OD 2** | 0,415 | 0,424 | 0,039 | 0,009 |
| **OD 3** | 0,416 | 0,426 | 0,041 | 0,009 |
| **OD Mean ±SD** | 0,416± 0,002 | 0,425± 0,001 | 0,040± 0,001 | 0,009± 0,001 |

**4. Recovery rate Ɵ**

Recovery rates after extractions and quantification are shown in **Table 3**. The average recovery rates of the first 10 extracts to the 10 th extracts are respectively 94,749 ± 0,716%; 4,610 ± 0.723%; 0.570 ± 0.098% and 0.071 ± 0.019%. A very good recovery rate (94.749 ± 0.716%) was obtained from the first extractions.

**Table 3: Recovery rate of extractions at different stages (%)**

| **Trial (n=10)** | **Rate 1 (%)** | **Rate 2 (%)** | **Rate 3 (%)** | **Rate 4 (%)** |
|---|---|---|---|---|
| **Mean Ɵ ± SD** | 94.749± 0.716 | 4.610 ± 0.723 | 0.570 ±0,098 | 0.071 ± 0.019 |

**5. The coefficient of variation (CV) on DO reading for each extract**

The coefficients of variation (CV) are presented in Table 4 below: The CV of extracts 1, 2, and 3 on OD reading are less than 5%, which reflects the fidelity of the method. While the CV of extract 4 is greater than 5%. This value (CV = 11.11) doesn't reflect the precision of the method on extract 4.

**Table 4: Coefficient of variation (CV) on OD reading**

| | **Extract 1** | **Extract 2** | **Extract 3** | **Extract 4** |
|---|---|---|---|---|
| **OD** | 0.418 | 0.425 | 0.040 | 0.010 |
| | 0.415 | 0.424 | 0.039 | 0.009 |
| | 0.416 | 0.426 | 0.041 | 0.009 |
| **OD Mean±SD** | 0.416 ± 0.002 | 0.425 ± 0.001 | 0.040 ± 0.001 | 0.009 ± 0.001 |
| **CV (%)** | 0.48 | 0.24 | 2.5 | 11.11 |

**6. Moisture of kola nuts**

Water content of the nuts before and after storage is shown in Table 5. Overall, the polystyrene (PS) trays have been permitted to maintain the freshness of kola nuts within six

months. In addition, the baskets were able to maintain the freshness of the nuts for four (4) months. As for the bag triple bagging, an increase in water content and rotting kola nuts were observed from the first month of storage. ANOVA variance analysis of nut water content at each month of storage shows a significant difference at the 5% level. The values of p are: 0.022; 0.005; 0.016; 0.015; 0.015; 0.022 and 0.025 respectively for months 0; 1; 2; 3; 4; 5 and 6. The highest water content (73.94% and 73.51%) was observed in the first month and this in the lots kept in the triple bagging bag. After 6 months of storage, the lowest water content (50.57%) was observed in the red nuts preserved in the rattan basket. While after 6 months of storage the highest values, namely 66.07% and 66.04% respectively are observed in the red and white nuts conserved in trays PS. There was, therefore, a large variation in the water content of kola nuts depending on the color, the type of packaging used and the storage time. PS trays have allowed better conservation of kola nuts compared to the rattan basket.

**Table 5: Evolution of the moisture content of kola nuts during storage**

| Treatments | Month 0 | Month 1 | Month 2 | Month 3 | Month 4 | Month 5 | Month 6 |
|---|---|---|---|---|---|---|---|
| **BPH-CBB** | 69.81±0.36$^{b}$ | 65.64±013$^{b}$ | 67.56±0.11$^{c}$ | 62.93±0.16$^{a}$ | 63.81±0.71$^{a}$ | 66.33±0.23$^{c}$ | 66.04±0.29$^{c}$ |
| **BPH-CBP** | 69.81±0.36$^{b}$ | 68.16±0.14$^{d}$ | 65.89±0.10$^{a}$ | 63.41±0.24$^{b}$ | 72.66±0.78$^{d}$ | 64.26±0.23$^{b}$ | 58.69±0.45$^{b}$ |
| **BPH-CRB** | 68.58±0.16$^{a}$ | 65.20±0.19$^{a}$ | 66.58±0.02$^{b}$ | 69.77±0.04$^{c}$ | 66.19±0.10$^{b}$ | 66.33±0.11$^{c}$ | 66.07±0.16$^{c}$ |
| **BPH-CRP** | 68.58±0.16$^{a}$ | 66.33±0.10$^{c}$ | 70.22±005$^{d}$ | 71.06±0.12$^{d}$ | 70.77±0.06$^{c}$ | 63.26±0.11$^{a}$ | 50.57±0.08$^{a}$ |
| **BPH-CBS** | 69.81±0.36$^{b}$ | 73.51±0.17$^{e}$ | - | - | - | - | - |
| **BPH-CRS** | 68.58±0.16$^{a}$ | 73.94±0.16$^{e}$ | - | - | - | - | - |

*Mean ± standard deviation, n = 3; the values of the same column being assigned the same letter are not significantly different according to the Tukey test at the 5% threshold.*

***BPH-CBB:*** *White kola nut conserved in Polystyrene Trays;* ***BPH-CBP:*** *White kola nut conserved in Rattan Basket;* ***BPH-CBS:*** *White kola nut conserved in triple bagging bag;* ***BPH-CRB:*** *Red kola nut conserved in Trays polystyrene;* ***BPH-CRP:*** *Red kola nut conserved in the Rattan Basket;* ***BPH-CRS:*** *Red kola nut conserved in the triple bagging bag.*

**7. Caffeine content of kola nuts**

Evolution of caffeine content of conserved kola nuts is summarized in **Figure 2.** Caffeine content varies from one color (red nut or white nut) to another depending on the type of packaging and the duration of conservation. In fact, at $T_0$, average caffeine contents were

7.74 ± 0.13 and 9.73 ± 0.10 g/kg, respectively for white nuts and red nuts. After one (1) month of storage, the caffeine contents were almost null in the nuts kept in the triple bagging bag. At the sixth month of storage, a rise in the caffeine content of 9.12 ± 0.09 and 12.28 ± 0.08 g/kg, respectively for white kola nuts and red walnuts was observed with the basket rattan. It should also be noted that from the third month, nuts stored in PS trays suffer a slight decrease in caffeine content.

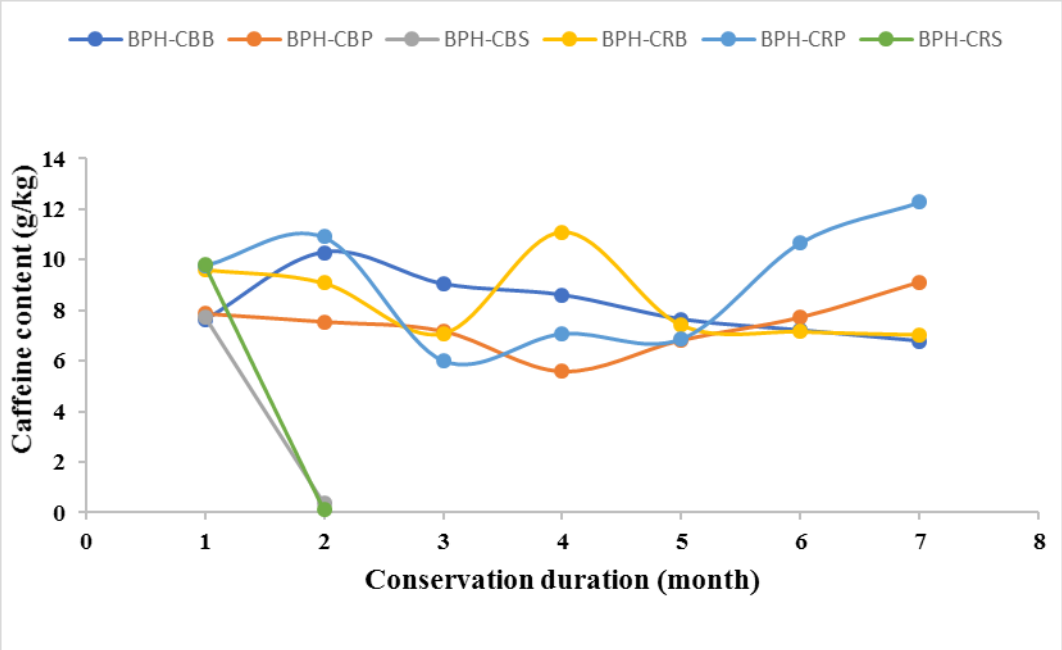


**Figure 2: Evolution of caffeine content during conservation according to the conditioning method**

***BPH-CBB:*** *White kola nut conserved in Polystyrene Trays;* ***BPH-CBP:*** *White kola nut conserved in Rattan Basket;* ***BPH-CBS:*** *White kola nut conserved in triple bagging bag;* ***BPH-CRB:*** *Red kola nut conserved in Trays polystyrene;* ***BPH-CRP:*** *Red kola nut conserved in the Rattan Basket;* ***BPH-CRS:*** *Red kola nut conserved in the triple bagging bag.*

**DISCUSSION:**

For optimal determination of the caffeine content of kola nuts, an assay method has been developed and validated for extraction and quantification by UV-Vis spectrophotometry. The objective of this validation is to produce reliable and reproducible results [18]. The validation of the analytical method is also one of the main requirements of the recognition of the competence of a laboratory according to [19]. This method of measurement allowed to follow the evolution of the caffeine content of kola nuts preserved in several containers. The validation of a method requires the choice of a suitable and effective solvent to ensure the

solubility and stability of the analyte which is in this case caffeine [18]. In this method, chloroform was used for the extraction of caffeine contained in kola nuts. According to [8], caffeine is very soluble in this solvent. This solvent thus made it possible to obtain a very good yield of extraction of caffeine from kola nuts with an average recovery rate of 94.749 ± 0.716%.

Furthermore, the method we developed clearly showed a good linear relationship between the absorbance and the concentrations of the standard solutions ($R^2$ = 0.9997) over a relatively wide concentration range from 0.003 to 0.01 g/l. This statistical measure shows that the regression line gives values very close to reality. The repeatability and reliability of the method expressed by a small value of the coefficient of variation (CV = 0.936 ± 0.589%) were excellent. Indeed, this CV is less than 5%, which is the limit value set by the Center of Expertise in Environmental Analysis of Quebec [15]. The method developed was able to detect caffeine from a concentration of 0.17 mg/l corresponding to the limit of detection (LOD). While the lowest concentration of caffeine that could be quantified (LOQ) with acceptable accuracy and precision was 0.57 mg/l. These LOQ and LOD values were sensitive enough to evaluate the caffeine content of the *Cola nitida* samples. Moreover, these values corroborate those found by [20] in a validation study of a caffeine dosing method using HPLC where the LOD was 0.13 µg/ml (0.13 mg/l) and the LOQ was 0.42 µg / mL (0.42 mg/l).

As for the moisture content of kola nuts, the highly variable values obtained are linked to the variety (color), the type of packaging used and the storage time. However, the moisture content of newly picked fresh nuts is 69.81 ± 0.36% and 68.58 ± 0.16% respectively for white nuts and red nuts. These values are much higher than those found by [21] where dry matter ranged from 50.82% to 55.24% (water content of 44.76% to 49.18%). This difference would be due to the origin of the nuts, the type of plant (tame or wild) and the method of drying. In fact, we used San Pedro nuts and oven dried at 105 ° C, contrary to [21] who used Agboville (Côte d'Ivoire) nuts and practiced drying at room temperature (30 ± 2 ° C).

During storage, maintaining the freshness of the nuts by the trays would be due to the fact that polystyrene trays and associated cartons are a barrier to oxygen and light. They limit the breathing and transpiration of the fruits. In addition, nuts kept in baskets lined with leaves of *Thaumatococcus daniellii* lost their freshness after 6 months. This result is explained by a loss of water from these nuts. Indeed, according to [22], too-permeable packaging will result

in a rapid loss of moisture, which will be accompanied by wilting and wrinkling of the product.

Caffeine is a secondary metabolite released by the kola tree in order to defend itself against external aggressions. Its content can vary according to the plants, the production areas, the color, and environmental conditions. In San Pedro, the caffeine content of freshly picked red walnuts is higher than that of white walnuts, ie 7.74 ± 0.13 and 9.73 ± 0.10 g / kg, respectively for white nuts and red nuts. Our results corroborate those found by [21] where the caffeine content of nuts ranged from 7,129.7 ± 3.06 to 10,812.5 ± 6.27 milligrams per kilogram of cola nuts depending on color. *Cola nitida* nuts contain 2-3% caffeine [23, 24].

The variation in caffeine and moisture content according to color is mainly due to the variation in the constitutive elements of kola nuts. Indeed, the white kola nut contains more total polyphenols and total flavonoids than the red nuts, taking into account the different extraction solvents [21]. While red nuts contain more caffeine than white nuts.

After six months of storage, when packaging is appropriate, the levels of water and caffeine content drop slightly as observed with PS trays. While when the packaging is inappropriate (case of rattan basket), after a period of 3 months of resistance, it loses its integrity and promotes a rapid loss of water in kola nuts. Thus, there is an increase in the caffeine content when uncontaminated nuts lose water. On the other hand, when the packaging is not suitable (in the case of triple bagged bags), the nuts cannot be stored for a month. There is a very strong sweating of the nuts which promotes their rot. Kola nuts are fruits with strong sweating and breathing. They are degraded by wind, low temperatures, and light.

Kola nut is a non-climacteric fruit and does not ripen after picking. Any post-harvest evolution of non-climacteric fruits is a degradation that results in a rapid deterioration of quality, caused by water loss, fruit senescence and/or fungal attack [25]. [3] studies showed that kola nuts were even better preserved at 29 ± 1 ° C than at 26 ± 1 ° C for rattan baskets, PVC trays and food carton used as primary packaging. Our results are in agreement with those of [3]. Indeed, opaque PS trays allow a better conservation of kola nuts. The rattan basket could be used, but a repackaging every three months is required to preserve the freshness and integrity of the kola nuts. Because, when the leaves of *Thaumatococcus daniellii,* lose their freshness as they age, they favor the circulation of the air and thus a direct contact with the nuts.

The methods of preserving the kola nut exposed and retained above make it possible, at a reasonable cost, to sell the colas purchased at harvest six months later. Their resale price can then be optimized according to the evolution of supply and demand. In fact, kola nuts stay fresh for more than six months.

## CONCLUSION:

In our study, the UV-Vis spectrophotometry method used for the quantification of caffeine contained in kola has proved relatively easy, fast and accessible. It does not require expensive solvents and reagents, it can be recommended for rapid, accurate and sensitive quantification of caffeine in kola nuts.

From this developed and validated method, the caffeine in the cola batches was assayed. The results obtained showed that *C. nitida* is a good source of caffeine and could, therefore, be used for the manufacture of new value-added products.

After six (6) months of storage of cola, the results show that the different packaging used and the shelf life have a significant influence on the water content and caffeine content of kola nuts. Of the three packages proposed, only one preserves the physicochemical properties of kola nuts for 6 months. These are opaque PS trays. This packaging can, therefore, be chosen for long-term storage of kola nuts.



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