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Validation of Efficiency Method for Heavy Metals Determination in Kola Nuts (*Cola nitida* Schott & Endl.) from Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. Author KKR designed the study, wrote the protocol, fitted the data and wrote the first draft of the manuscript. Author NY checked the first draft of the manuscript and achieved the submitted manuscript. Authors KY, KC, CA and SD performed the statistical analysis and assisted the experiments implementation. Author BH expertized the results interpretations. All authors managed the literature, read and approved the submitted manuscript.

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ABSTRACT

Aims: The current study targets the achievement of a reliable process for the determination of heavy metal contents in kola nuts, namely cadmium, mercury, and lead, for better appreciation of the risks incurred from the consumption of such food products.

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Study Design: kola nuts collected from different stakeholders (planters, collectors, stores and centers) were analyzed after the validation of the proposed analytical method.
Place and Duration of Study: Central Laboratory for Food Hygiene and Agro-Industry, LANADA in Abidjan, Côte d'Ivoire, running 2018.
Methodology: Two references were used for the validation of the analytical method, namely the French standard NF V 03-110 and the European directive 2001/22/EC. The assays were achieved with an flame Atomic Absorption Spectrophotometer (AAS). The heavy metal contents of some samples collected from different sampling place were then determined
Results: From the data, a significant regression chart was recorded for the heavy metals detection graphs, with significant correlation coefficients (R^2> 0.99). The linearity domain was validated between 0.5 µg/L and 1.5 µg/L for cadmium, 15 µg/L and 45 µg/L for lead and from 10 µg/L to 100 µg/L for mercury. In addition, the LOD were 0.03 µg/L, 1.85 µg/L and 2.92 µg/L, while the LOQ were 0.07 µg/L, 6.52 µg/L and 3.32 µg/L for cadmium, lead and mercury, respectively. The relative standard deviations of the repeatability and reproducibility assays are below 4%, whereas standard additions of heavy metals are fully recovered, with percentages close to 100%. Contents of cadmium, lead and mercury in kola nuts are respectively valued at 22.97±9.01 µg/kg, 1065.57±613.76 µg/kg and 33.88±31.58 µg/kg from the farmers and 24.99±7.79 µg/kg, 296.51±98.18 µg/kg and 39.74±34.66 µg/kg from the storage centres.
Conclusion: This analytical method could help in ensuring effective sanitary control at different critical points of kola nut distribution channel for promoting a good management of the toxicity concerns in such products.

Keywords: Cola nitida; heavy metals; cadmium; lead; mercury; validation method.

1. INTRODUCTION

Native from the tropical forests of Western Africa, *Cola* genus (*Sterculiaceae*) includes about 40 species and the nuts for the commercial extract are derived, almost exclusively, from two species of Cola, either *Cola nitida* (Vent.) Schott and Endl. or *Cola acuminata* (Beauv.) Schott and Endl [1,2,3]. In Côte d'Ivoire, the most common cola crops are from *Cola nitida* species generally grown in the districts of the Mountains, Comoe, Lagoons and Down-Sassandra [4]. The cola plant greatly grows with annual rainfall over 1000 mm and on soft and well-drained soils [5]. The main interest of the cola crop lies in the production of fruits known as kola nuts.

According to Asogwa [6], one of the major constraints of the cola cultivation lies in the infertility of the soils. So, for improving the yield, numerous farmers usually use chemical fertilizers and pesticides [7] which can reduce both the plant's strength and the fruits quality in long-range. Of course, this practice really succeeds in improving the growth and yield of the plant [8]. Unfortunately, it also brings toxic elements into the crops [9].

Otherwise, the post-harvest preservation of the raw crops is another significant constraint for the cola stakeholders [1,10]. Indeed, kola nuts are generally consumed fresh [11]. Yet, the fresh crops state easily allows proliferation of microbes, ants and other parasites. In order to control the crops post-harvest enemies and to keep the fruits fresh, the farmers and traders generally soak the raw kola nuts in organic pesticides solutions [12,13].

The use of chemicals in the cola sector is observed in the planted soils and during the crops carriage and processing. Indeed, the kola nuts distribution channel is generally from farmers to the big storage, processing and export centres, with temporary stay from rural collectors and small urban stores [4]. During their processing, carriage and sale, heavy metals could be laid on the kola nuts stock [14]. According to Nordström [15] and Vine [16], the use of pesticides results in negative impact on the environment and human health. Furthermore, the works of Biego et al. [13] and Aikpokpodion et al. [3] showed the presence of organochlorine pesticides in kola nuts at concentrations over the maximal values admitted by the *Codex Alimentarius*.

According to Adeosun et al.[8], 90% kola nut production is daily consumed by the populations during native ceremonies such as weddings, baptisms, friendly meeting, funeral and the sacrifices rituals [17,18,19]. This high consumption is due to the alleged properties of nuts in particular stimulation of the nervous system, energy, and dietary properties [20,21]. Some Studies highlighted correlations between

such properties and the large amount of alkaloids, polyphenolics and carbohydrates compounds in kola nuts [22,23]. However, accounting the various anti-nutrient substances as pesticides, mycotoxins, and heavy metals also found in kola nut, the consumption of this raw product is a source of public health toxicity concerns [13,24].

The heavy metals, namely lead (Pb), cadmium (Cd), and mercury (Hg) are known as strict and toxic contaminants for living beings, even at very lower concentrations [25,26]. The accumulation in the food chain is one of the harmful properties of these heavy metals. They reach foods led by the air, household and industrial waste, animal dung and fertilizers [27]. The permanent exposure of the human being to lower measures of these heavy metals is reported to be co-factor of some neurological, carcinogenic and digestive diseases [28]. In addition, they represent the third source of food risk for human and animal after mycotoxins and microorganisms [26].

For the consumer's health, the European Commission worked about a regulation laying down the maximum limits of heavy metals residues in fruits. From the resulted standard, the maximal values agreed are 0.05 mg/kg for cadmium and 0.5 mg/kg for mercury and lead [29]. However, international trades involve the systematic control of foodstuffs to insure their safety. In order to promote the distribution and consumption of foodstuffs, the analysis and control methods regarding toxic substances need to be effective and sound. The current study exhibits and validates a running method for the sure assessment of cadmium, lead, and mercury in kola nuts from Côte d'Ivoire.

2. MATERIALS AND METHODS

2.1 Sampling and Pre-treatment

Sampling was achieved in accordance with the Regulation N° 333/2007of the European Commission [30]. Thus, 2 kg of kola nuts samples were collected in each district: Mountains, Comoe, Lagoons, and Down-Sassandra. The samples precisely derived from farmers, rural collectors, urban stores and big storage centres. Kola nuts were cut into small pieces with clean stainless knife. Then, they were dried at room temperature (30 ± 2ºC) for four weeks away from sun light ground in a hammer mill and kept in polyethylene sealed bags before their achievement at Central Laboratory for Food Hygiene and Agro-Industry (Abidjan, Côte d'Ivoire) to be analyzed.

2.2 Reagents

All reagents used in this study were of pure analytical grade, unless otherwise specified, were purchased from Merck, Germany : nitric acid 65%,hydrogen peroxide 35%, tin II chloride, steaming hydrochloric acid 37%, standards of cadmium, lead and mercury and ultrapure water at 18 MW.

2.3 Apparatus and Conditions of Quantification of Heavy Metals

An Atomic Absorption Spectrophotometer (AAS, type VARIAN SPECTRAA 110) with GTA 110 furnace was used for the determination of cadmium and lead. Regarding the determination of mercury, the AAS was equipped with a VGA77 vaporization unit in the presence of a solution of 10% tin-II chloride previously prepared with 37% fuming hydrochloric acid. Nitrogen was used as a vector gas for the analysis. The operating conditions of the AAS device are shown in Table 1.

2.4 Validation of Analytical Method

The validation of this analytical method for the determination of heavy metals (cadmium, lead, and mercury) was performed according to the French Standard (AFNOR, NFV03-110-1998) and the European directive 2001/22/EC [31, 32].The process includes the study of the linearity for the calibration range, the determination of the limits of detection and quantification (LOD and LOQ values), the calculation of the relative standard deviation regarding repeatability and reproducibility assays, and the calculation of the recovery percentage for the analysis accuracy essays.

2.4.1 Evaluation of the linearity

The adequacy of the calibration curve to the linear design was examined using 5 replications of a 5 independent points range. The linearity was assayed including the working range. The 5 calibration points were:

- 0.5 µg/L, 0.8 µg/L, 1 µg/L, 1.2 µg/L, 1.5 µg/L for cadmium
- 15 µg/L, 20 µg/L, 25 µg/L, 30 µg/L, 45 µg/L for lead;
- 10 µg/L, 25 µg/L, 30 µg/L, 50 µg/L, 100 µg/L for mercury.

Table 1. Operating conditions of the atomic absorption spectrophotometer

	Cadmium	Lead	Mercury
Current intensity (mA)	4	10	4
Width of the slot (mm)	0.5	1	0.5
Wavelength (nm)	228.8	217	253.7
Coefficient of variation (%)	1	1	1
Integration time (seconds)	5	5	5
Number of repetitions	3	3	3

2.4.2 Limits of detection and quantification

The limits of detection (LOD) and quantification (LOQ) were calculated from the analysis of 10 separate assays of blank matrices. These parameters were measured using the following formulas:

LOD=Mx+ 3S
LOQ =Mx+ 10S

With:

LOD:Limits of detection,
*LOQ: L*imits of quantification*,*
Mx: Average from 10 assays of blank matrices,
S: Standard deviation of blank values.

2.4.3 Assessment of the repeatability and reproducibility

The repeatability of the analysis was probed with 10 assays of reference sample. For the reproducibility, 5 separate assays were achieved with the reference sample at several days intervals.

2.4.4 Analysis of the recovery

The extraction rate was determined from addition of various standards concentrations of heavy metals to uncontaminated solutions of kola nut samples. The concentrations of the added standard were 0.018 mg/kg, 0.116 mg/kg and 0.044 mg/kg for cadmium, lead and mercury, respectively. Ten separate assays were achieved to assess the recovery rate allowed by the method of heavy metal determination.

2.5 Method of Heavy Metals Mineralization

An Aliquot of 0.5 g homogenate of each sample was heat mineralized with 7 ml of concentrated nitric acid (65%) and 1 ml of hydrogen peroxide (35%) using a microdigest for 20 min [33]. The mineralizate was reduced with a 10% chloride tin II solution previously prepared with 37% steaming hydrochloric acid for the mercury [34]. The resulted mineralised was then diluted in high quality ultrapure water and investigated with Atomic Absorption Spectrophotometry.

2.6 Statistical Analysis

The data were statistically treated using Statistical Program for Social Sciences (SPSS 20.0, SPSS for windows, USA) at 5% significance. Mean concentrations of cadmium, lead and mercury were calculated; then the relative standard deviations were used as values of repeatability and reproducibility. The square of Pearson correlation coefficient (R^2) was calculated to appreciate the linearity. The recovery rate was estimated to express the extraction yield. The mean concentrations and the concentrations' variation range of the heavy metals allowed the description of contamination range of the cola samples. The comparison of the concentrations recorded with the reference values was performed using other 5% risk of conformity test.

3. RESULTS

3.1 Validation Parameters for the Quantification of the Heavy Metals

The validation data deal with the values of linearity, repeatability, reproducibility, soundness, and limits of detection and quantification involved from the heavy metals determination.

The results of the linearity analysis are recorded in Table 2. All the analytes exhibited good linearity over the evaluated range with significant correlation coefficients ($R^2 > 0.99$).

Table 3 displays the statistical validity of the linearity over the full calibration range according to the statistical Fisher rule. Indeed, the F1 values calculated for regression (3743, 5758 and 6365 for Cd, Pb and Cd, respectively) are higher than the critical Fischer value (8.10). On the other hand, the F2 values are calculated for the error trend (2.97, 4.22, and 4.66 for Cd, Pb, and Hg, respectively) are lower than the critical Fisher value (4.94).

Table 2. Calibration equationand determination coefficient from the heavy metal assessment

Heavy metal	Calibration equation[a]	Coefficient of determination (R^2)
Cadmium (Cd)	y = 0.1836x + 0.0274	0.9978
Lead (Pb)	y = 0.0182x + 0.0949	0.9983
Mercury (Hg)	y = 0.0011x - 0.0021	0.9981

[a]: *y – absorbance; x – concentration (µg/L)*

Table 3. Linearity traits deriving from the experimental domain calibration

Heavy metals	F1	Critical F-value	Rating of the regression trend	F2	Critical F-value	Calibration domain
Cadmium (Cd)	3743		Acceptable	2.97		Acceptable
Lead (Pb)	5758	8.10	Acceptable	4.22	4.94	Acceptable
Mercury (Hg)	6365		Acceptable	4.66		Acceptable

F1: $F_{\text{-value}}$ for the regression trend; F2: $F_{\text{-value}}$ for the statistical error trend

Values of the limits of detection (LOD) and quantification (LOQ) of the heavy metals assessed are showed in Table 4. Values reported are 0.03 µg/L, 1.85 µg/L, and 2.92 µg/L for cadmium, lead, and mercury, respectively. Whereas the LOQ values are 0.07 µg/L (Cd), 6.52 µg/L (Pb) and 3.32 µg/L (Hg).

The relative standard deviation (RSD) calculated from the repeatability assays are 3.32%, 2.11%, and 2.74% for cadmium, lead and mercury, respectively. Regarding the reproducibility, the RSD values are 3.98%, 3.28%, and 3.33% for respective cadmium, lead and mercury (Table 5).

The mean extraction yields resulting from the recovery of the measures added compared to the standard recovery of the heavy metals studied are 97.72% (Hg), 102.78% (Cd), and 104.31% (Pb) as showed in Table 6.

3.2 Trends of Heavy Metals Concentrations in Cola Samples

Table 7 shows the variation of the heavy metals concentrations of the cola samples from farmers and big storage centres. Values are reported on the dry matter basis.

Table 4. Minimal concentration (µg/L) for the detection and the quantification of the heavy metals from the determination method used

Heavy metals	Limit of detection	Limit of quantification
Cadmium (Cd)	0.03	0.07
Lead (Pb)	1.85	6.52
Mercury (Hg)	2.92	3.32

The mean concentrations of heavy metals from the farmers were 22.97 µg/kg, 1065.57 µg/kg, and 33.88 µg/kg for cadmium, lead, and mercury, respectively.

From the big storage centres, the kola nuts record means of 24.99 µg/kg, 296.51 µg/kg and 39.74 µg/kg of cadmium, lead, and mercury, respectively.

The cumulative mean concentrations of heavy metals for the overall samples studied are displayed in Fig. 1.The highest content was found from the farmers (1122.42 µg/kg); whereas the big storage centers showed the lowest heavy metals cumulative content (660.75 µg/kg).

Table 5. Values measured (µg/L) and relative standard deviation (%) from the investigation of the repeatability and reproducibility during the determination of heavy metals assessed

Heavy metals	Repeatability			Reproducibility		
	Standard solution (µg/L)	Value measured	$RSD_{\text{-value}}$	Standard solution (µg/L)	Value measured	$RSD_{\text{-value}}$
Cadmium	0.8	0.78 ± 0.2	3.32	0.8	0.77 ± 0.03	3.98
Lead	30	30.72 ± 0.65	2.11	10	10.5 ± 0.34	3.28
Mercury	15	14.94 ± 0.41	2.74	15	14.19 ± 0.47	3.33

RSD-value: value of the relative standard deviation

Table 6. Data of the recovery traits for the heavy metals studied

Heavy metals	Standard recovery (mg/kg)	Recovery value measured (mg/kg)	Recovery rate (%)
Cadmium	0.018 ± 0.001	0.0185 ± 0.002	102.78 ± 3.7
Lead	0.116 ± 0.006	0.121 ± 0.009	104.31 ± 4.3
Mercury	0.044 ± 0.002	0.043 ± 0.003	97.72 ± 5.1

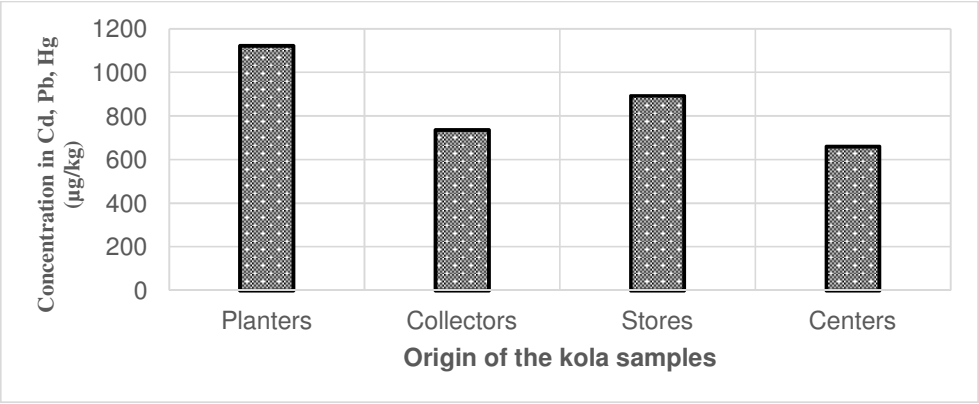

Fig. 1. Cumulative mean concentrations of three heavy metals from the kola nuts studied

Table 7. Mean kola nuts concentrations (µg/kg dry matter) in heavy metals

Heavy metals	Farmers		Bigstorage centres	
	Mean±SD	[min-max]	Mean±SD	[min-max]
Cadmium	22.97±9.01	3.35-33.80	24.99±7.79	3.49-36.16
Lead	1065.57±613.76	465.49-2421.93	296.51±98.18	133.37-3270.08
Mercury	33.88±31.58	nd-81.35	39.74±34.66	nd-108.42

SD: standard deviation; min: lowest value; max: highest value

4. DISCUSSION

The linearity of every determination method is the ability, within a range of digits, to provide information values or results which are proportional to the amount of the analyte to be measured from the material studied [35]. The assays performed for the linearity highlighted the normality of the distribution across the calibration range set at 0.5 µg/L to 1.5 µg/L, 15 µg/L to 45 µg/L and 10 µg/L to 100 µg/L for cadmium, lead and mercury, respectively. According to the current linearity data, the method for determining heavy metals (Cd, Pb and Hg) could be considered as a reliable process for kola samples.

From the statistical Fischer test, the results showed that the variance proportion due to the error of the experimental design is not higher than the variance of the experimental error. For each heavy metal measured, the calculated F_{value} is lower than the critical F_{value} corresponding to a Fisher variable at 1% statistical significance. The results obtained show that the linearity domain is valid and the regression design is also acceptable. In addition, overall Pearson determination coefficient s(R^2) recorded during the study were closed to 1.The determined second-order mathematical models are therefore valuable for forecasting the main responses [36].

The limits of detection (LOD) and quantification (LOQ) are similar to the values reported by Labat et al. [37]. These authors showed LOD values reaching 0.02 µg/L for the cadmium and 1.3 µg/L for the lead; whereas the LOQ values were respectively recorded at 0.03 µg/L and 1.4 µg/L. However, the slight variations of LOD and LOQ values could be explained by the soundness of the apparatus. Indeed, these authors have used the inductively coupled plasma added with a Mass Spectrometry (ICP-MS). According to Alsac [38], some chemical elements result in higher LOQ values in ICP-MS compared to the ICP-AES used in the current study.

Regarding the accuracy of the determination method as estimated by repeatability and reproducibility essays, overall relative standard deviations are lower, below 5%. The RSD-values were ranged from 2.11% to 3.32% for the repeatability and from 3.28% to 3.98% for the reproducibility. This observation is in the same trend as the work of El Alami [39] stating that the lower experimental error involved by standard deviation reflects the closeness between the values obtained from various measurements of the same object under specified conditions. Both reproducibility and repeatability analyses performed using our experimental design are sounded.

For the recovery assessment, the results showed recovery rates between 97.72% and 102.78% from the reference sample. In addition, there wasn't any significant difference with the evaluation of the conformity.

The data of this study are in accordance with the operating conditions recommended by the FAO [40] as acceptable analysis technique for heavy metals determination, since the results highlighted reliability and good precision of the mineralisation and analysis operations.

Thereafter, the kola nuts samples recorded significant contents in heavy metals (Cd, Pb, and Hg). The results evidenced various contents according to the origin of the kola sample and the heavy metal assessed. From overall cumulated heavy metals concentrations, the samples originating from the farmers recorded the highest value. The high concentrations of heavy metal in kola nuts can be attributed to the cultivation techniques used by farmers. However, the excessive use of chemical fertilizers is a source of contamination of agricultural soils and kola nuts [7]. Also, during bush fires, heavy metals are emitted into the environment as particles during combustion and contaminate kola nuts [41]. According to Dauguet [26], the changes of heavy metals concentrations in food stuffs derives from natural atmospheric conditions (volcanism, dust of erosion), anthropogenic activities (industry, transport), and human contributions (fertilizers, phytosanitary products, animal dung , urban sludge, etc.).

5. CONCLUSION

The study showed that the method suggested for the heavy metals determination from kola nuts is really suitable. The assays resulted in linear calibration curve within the heavy metals concentration range considered. The determination method is reproducible and repeatable, and is therefore trusted. It is easily implementable in every laboratory equipped with Atomic Absorption Spectrophotometer. It's also sensitive and does reveal any matrix effect (good specificity). Thus, this method could help in ensuring effective sanitary control at different critical points of kola nut distribution channel for promoting a good management of the toxicity concerns in such products.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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