



Optimization of Polyphenols Extraction Method from Kola Nuts (*Cola nitida* Vent. Schott & Endl.) Using Experimental Design

Yves B. Nyamien^{1,2*}, Olivier Chatigre¹, Emmanuel N. Koffi³, Augustin A. Adima², and Henri G. Biego¹

¹Laboratory of Biochemistry and Food Science, Training and Research Unit of Biosciences, Felix HOUPHOUËT-BOIGNY University of Abidjan, 22 BP 582 Abidjan 22, Côte d'Ivoire.

²Laboratory of Water Chemistry and Natural Substance, Training and Research Department of GCAA, Felix HOUPHOUËT-BOIGNY National Polytechnic Institute, BP 1093 Yamoussoukro, Côte d'Ivoire.

³Laboratory of Bioorganic Chemistry and Natural Substance, Training and Research Unit of Applied Fundamental Science, Nangui ABROGOUA University of Abidjan, 02 BP 801 Abidjan 02, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration between all authors. Author YBN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors OC, ENK, AAA and HGB managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BBJ/2015/17030

Editor(s):

(1) Anil Kumar, School of Biotechnology, Devi Ahilya University, Madhya Pradesh, India.

Reviewers:

(1) Nii Korley Kortei, Department of Nuclear Agriculture and Radiation Processing, Graduate School of Nuclear and Allied Sciences, University of Ghana, Ghana.

(2) Anonymous, Croatia.

Complete Peer review History: <http://www.sciencedomain.org/review-history.php?id=1041&id=11&aid=8646>

Original Research Article

Received 23rd February 2015
Accepted 16th March 2015
Published 1st April 2015

ABSTRACT

Aims: The aim of this study is to optimize extraction process of phenolics compounds from kola nuts by using experimental design.

Study Design: Kola nuts were collected in October 2014-February 2015 in south of Côte d'Ivoire. Harvested kola nuts were transferred to the laboratory until used in the experiments

Place and Duration of Study: This study was carried out during season 2014-2015 in the Laboratory of Biochemistry and Food Science, Félix Houphouët-Boigny University, Côte d'Ivoire.

*Corresponding author: Email: nyams02@gmail.com;

Methodology: Nuts were divided into two groups and subdivided to obtain four groups according to their variety (traditional or improved) and morphotype or cultivar (white and red). After drying and powder processing, the effect of six parameters (solvent type, solid-liquid ratio, extraction mode, variety, cultivar and extraction time) on polyphenol extraction from kola nuts were studied. Firstly, a Plackett-Burman design (8 experiments) was used to highlight the most important factors which influence the extraction process. Then, a full factorial design (2^k , $k=4$) was used to optimize the extraction conditions.

Results: Results showed that solvent, ratio (w/v), extraction mode and variety of nuts had significant effect on polyphenols extraction. The predicted optimal conditions for the highest polyphenol content from kola nuts were found with infusion of traditional variety at 1/100 (w/v) ratio with aqueous ethanol 50% (v/v). In the predicted optimal conditions, experimental values were 350 mg/L GAE, 1460 mg/L QE and 264.33 mg/L CE for total polyphenol, total flavonoid and condensed tannin, respectively. Experimental data were very close to the predicted values.

Conclusion: The extractive capability of kola nuts polyphenol is considerably depended on the solvent type, the extraction mode, the solid-liquid ratio (w/v) and nuts variety. Thus, kola nuts can be considered as a natural source of phenolics compounds with good antioxidant capacity. This optimization of the extraction parameters of phenolics compounds from kola nuts is very original, it is the first on a current scale of research on kola nut.

Keywords: *C. nitida*; polyphenols; optimized extraction; full factorial design.

1. INTRODUCTION

Natural antioxidants are increasingly appreciated by consumers due to both their inherent positive effects and to the possibility of using them as a source of natural additives to replace synthetic ones [1,2]. They are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischaemia, asthma, arthritis, inflammation, neurodegeneration, etc. [3]. The search of new antioxidants and phenolics from natural herbal source has taken very large attention in last decade [4]. Antioxidant activity of fruits and vegetables is generally positively correlated with their content of polyphenols [5]. Polyphenols are widely distributed in plants with antioxidant and antiradical properties [6,3], although they are found in relatively high amount in some plants, seeds and fruits [7] and beverages [8]. They have been studied by reserchers for their strong antioxidant health benefits [9-11].

Natural and secondary metabolic substances, especially, polyphenols, represent a wide range of substances with various structures, fall into different families including anthocyanins, coumarins, lignins, flavonoids, tannins, quinones, acids and phenols [5,6]. This structural diversity results in large variability of the physico-chemical properties influencing polyphenol extraction. Experimental data *in vitro* suggest that polyphenols have anti-inflammatory, antiallergic, anti-viral, and anti-carcinogenic activities [12-14]. They also have a protective role against chronic

degenerative diseases (cataracts, macular degeneration, neurogenerative diseases), cancer cardiovascular diseases [13].

Over the years, several assay methods, solvents and sample preparation techniques have been adopted for the quantification of polyphenolics but there is no universal extraction procedure suitable for extraction of all plant phenolics [15]. Extraction methods (traditional methods, ultrasound-assisted extraction, subcritical water extraction, supercritical fluid extraction, pressurized fluid extraction, or accelerated solvent extraction), particle size, sample preparation, extraction time, solvent type, temperature of extraction and the presence of interfering factors have been shown to strongly influence polyphenol extractability in plant materials [16-19].

Kola nuts is a natural plant that is a rich source of natural polyphenols and provides a high free radical scavenger activity [20-23]. There are over 140 species of kola nut trees, and the most commonly edible are bitter cola (*Garcina kola*), kola nuts (*C. nitida* or *C. acuminata*) [20,24,25]. These three species are used as stimulants, increasing energy and strenght, dispelling drowsiness and staving hunger [26,27,22]. But only *C. nitida* is one of great interest because locally cultivated, widely consumed when fresh, while the dried nuts are used for beverages and pharmaceutical purposes in Europe and North America [21,18].

Many parameters have significantly influenced the extraction yield of kola nuts. In order to extract the bioactive polyphenolic compounds from *Cola nitida* nuts, an experimental design with a Plackett-Burman design following by a full factorial design was carried out. We have used firstly the screening designs (Plackett-Burman design) which are best known for factors two levels. The experimentation highlights in a large number, those factors that are actually influential on a process in a fixed experimental field [28-29]. The choice of screening design is based on the number of the studied factors which is six (extraction method, extraction solution, solid-liquid ratio (w/v), extraction time, color and variety of nuts). The most important factors acting on bioactive compounds extraction were used in a second experimental design, full factorial design, that to examine the interactions effects of the factors on a response or dependent variable by carry out all possible combinations of levels and variable [30].

According to the literature, no work has so far been reported on the optimization of extraction of bioactive compounds from Côte d'Ivoire kola nuts. Under this situation a statistical method of optimization seems to be very useful. The objective of the present study was to optimize extraction conditions by using non-toxic solvent for the enhanced recovery of polyphenols from kola nuts. Plackett-Burman or Hadamard experimental design allowed the screening to identify the most important factors among ratio, extraction method, solvent type, extraction time, nut variety and morphotype or cultivar [29]. After this screening, the optimization was done by full factorial design while keeping in mind the factors that influence the extraction along with the effects of interaction between these factors [31].

2. MATERIALS AND METHODS

2.1 Plant Material

A fresh kola nut was used as plant material. They were collected from October 2014 to February 2015 in south of Côte d'Ivoire.

As shown in Table 1, harvested plant materials were organized into two groups and subdivided to obtain four groups according to their color or cultivar and their variety. Two varieties of nuts

are available in Côte d'Ivoire: nuts did not undergo genetic modification (Traditional or spontaneous nuts) and those from the breeding program initiated by the National Agricultural Research Centre (improve nuts). At laboratory, they were washed with distilled water, cut into smaller pieces and dried at room temperature ($30\pm 2^\circ\text{C}$) during two weeks. The dried sample was milled into powder using an electric blender, and stored in plastics bags prior to analysis.

Table 1. Sampling of kola nuts

Sample identification	Fresh nuts color	Variety
RCN ₁	Red	Traditional
RCN ₂	Red	Improve
WCN ₁	White	Traditional
WCN ₂	White	Improve

RCN : Red *C. nitida*, WCN : White *C. nitida*

2.2 Chemical Reagent

All reagents used in the study were of pure analytical grade, unless otherwise specified. Ethanol ($\text{CH}_3\text{CH}_2\text{OH}$), methanol (CH_3OH), hydrochloric acid (HCl), potassium peroxodisulfate ($\text{K}_2\text{S}_2\text{O}_8$), sodium nitrite (NaNO_2), Folin-Ciocalteu's phenol reagent, sodium carbonate salt (Na_2CO_3), sodium hydroxide (NaOH) and aluminum chloride (AlCl_3) were purchased from Carlo Erba (Spain). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid, quercetin, catechin were purchased from Sigma-Aldrich (Germany). ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt) was purchased from Biochem (France). Vanillin was purchased from Merck (Germany). Water was purified by a Milli-Q water purification system.

2.3 Plackett and Burman Design

The Plackett-Burman experimental design was used for screening the effect of six variables which include extraction solution (X_1), Ratio (X_2), extraction mode (X_3), morphotype or cultivar (X_4), variety (X_5) and extraction time (X_6) on the extraction from kola nuts. These six independent variables or factors were organized in eight combinations according to Plackett-Burman design matrix [29]. For each factor, a high (+1) and low (-) level were tested (Table 2).

Table 2. Plackett-Burman parameters and coded levels

Factors	Technological parameters	Coded levels	
		Low (-1)	High (+1)
X ₁	S: Extraction solution	Water	Ethanol 50%
X ₂	R: Ratio (w/v)	1/100	5/100
X ₃	M: Extraction mode	Maceration	Infusion
X ₄	C: Morphotype or cultivar	White	Red
X ₅	V: Variety	Traditional	Improve
X ₆	T: Extraction time (h)	3	24

The responses studied were total phenolics content (TPC), total flavonoids content (TFC) and condensed tannin content (CTC). The model created from this analysis of Plackett-Burman experimental design using multiple regression analysis is based on the 1^{er} order-model:

$$Y_n = b_0 + \sum b_i X_i$$

Where Y_n is a predicted response, b₀ is a model constant and b_i is a variable linear coefficient.

2.4 Full Factorial Design

A 2⁴ full factorial experimental design was used to identify the relationship existing between the response functions and process variables [32], as well as to determine those conditions that optimized extraction of TPC, TFC, and CTC. The four independent variables or factors studied were the extraction solution (X₁), ratio (X₂), extraction mode (X₃) and variety (X₅). Each variable to be optimized was coded at the lower (-1) and higher (+1) levels considered as previously studied (Table 3).

Table 3. Experimental values and code levels of independent variables used for the 2⁴ factorial designs

Factors	Technological parameters	Coded levels	
		Low (-1)	High (+1)
X ₁	S: extraction solution	Water	Ethanol 50%
X ₂	R: ratio (w/v)	1/100	5/100
X ₃	M: extraction mode	Maceration	Infusion
X ₅	V: variety	Traditional	Improve

In the full factorial design, the main as well as the interaction effects of various factors are determined by fitting the data into 1^{er} order polynomial equation:

$$Y_n = b_0 + b_1 X_1 + b_2 X_2 + \dots + b_k X_k + \dots + b_{12} X_1 X_2 + \dots + b_{k-1k} X_{k-1} X_k + \dots + b_{1\dots k} X_1 X_2 \dots X_k$$

Where Y_n was the measured response, b_k the main effect of the factors X_k, b_{k-1k} the interaction effect between the factors k-1 and k and b₀ the constant term.

2.5 Extraction Procedure

Every dried sample (1 or 5 g) was extracted by diffusion with 100 ml of the extraction solution (water or ethanol 50%) according to the two methods (maceration or infusion) selected. The mixture was least for stand at room temperature during extraction time (3 h or 24 h). Extracts obtained were filtered through a filter paper (Whatman N°1) and stored at 4°C in refrigerator for subsequent determination.

2.6 Analytical Methods

2.6.1 Determination of total polyphenols content (TPC)

Total polyphenols were determined by colorimetry, using the Folin-Ciocalteu method [33,34]. Diluted Folin-Ciocalteu reagent (1/10, v/v, 2.5 mL) was added to 30 µL of sample. After 2 min of incubation in the dark at room temperature, 2 mL of aqueous sodium carbonate (75 g/L) was added. After gentle stirring, the mixture was incubated in a water bath at 50°C for 15 min and rapidly cooled down to stop the reaction. The absorbance was measured at 760 nm with distilled water as blank. A calibration curve was performed with gallic acid at different concentrations (0-1 g/L). Analyses were performed in triplicate and polyphenols level was expressed in milligrams gallic acid equivalent per liter of extract (mg/L GAE).

2.6.2 Determination of total flavonoids content (TFC)

Total flavonoids were determined by the aluminum chloride colorimetric method described by Marinova et al. [35]. In a 25 mL volumetric flask, 0.75 mL of sodium nitrite (NaNO₂) distilled water solution (5%, w/v) was added to a 2.5 mL

aliquot of the sample. The color reaction was left to develop for 5 min in the dark and at room temperature. Then, 0.75 mL of AlCl_3 distilled water solution (10%, w/v) was added and incubated for 6 minutes. After incubation, 5 mL of sodium hydroxide (NaOH 1M) were added and the volume made up to 25 mL. The mixture was mixed well before being dosed with UV-Visible spectrophotometer. The reading was taken at 510 nm with distilled water as a blank. A calibration curve was performed with quercetin at different concentrations (0-1 g/L). The tests were performed in triplicate and the flavonoids content was expressed in milligrams quercetin equivalent per liter of extract (mg/L QE).

2.6.3 Determination of condensed tannins content (CTC)

Condensed tannins were determined by the method of Heilmer et al. [36]. 400 μL of each extract was added to 3 mL of a methanol solution of 4% vanillin and 1.5 mL of concentrated hydrochloric acid was subsequently added. After 15 min of reaction the absorbance was measured at 550 nm with distilled water as blank. A calibration curve was performed with catechin at different concentrations (0-500 $\mu\text{g/mL}$). The tests were performed in triplicate and the tannins content was expressed in micrograms catechin equivalent per milligram of extract (mg/L CE).

2.7 Statistical Analysis

All experiments were done in triplicate and data in tables and figures represent mean values \pm standard deviation ($n=3$). Coefficient and experimental standard deviations were determined by the method of linear regression (MS Excel 2007). Comparison of mean values of measured parameters was performed by a one-way ANOVA (STATISTICA, version 7.1) using post hoc Low Statistical Difference (LSD) test. The mean values were considered significantly different when $P=.05$.

3. RESULTS AND DISCUSSION

3.1 Standards Parameters

The absorbance values of stocks solutions of standard gallic acid, quercetin and catechin for Total Polyphenol (TP), Total Flavonoid (TF) and Condensed Tannin (CT), respectively were measured. Table 4 shows the different equations of each standard linear calibration and

their regression coefficient R^2 . It shows clearly a good linear relationship between the absorbance and concentration of each standard solution ($0.994 < R^2 < 0.997$). Values give a good agreement between the experimental and predicted values of the adapted model [37,38].

Table 4. Equation and regression coefficient for different standard calibration

Standards	Equation	R^2
Gallic acid	$y=0.872x - 0.006$	0.996
Quercetin	$y=0.644x - 0.006$	0.997
Catechin	$y=0.002x - 0.035$	0.994

3.2 Screening of Variable Effects on Polyphenols Extraction

According to Plackett-Burman experimental design, 8 experiments were carried out in order to evaluate the effects of the main factors on total phenolics, total flavonoids and condensed tannins extractions. In Table 5, the factors X_1 , X_2 , X_3 , X_4 , X_5 and X_6 represent extraction solution, ratio (w/v), extraction mode, morphotype or cultivar, variety and extraction time (h), respectively. The higher level variable was designed as (+1) and the one at the lower level as (-1).

The experimental analysis showed that the yield extraction is favored when the variables extraction solvent, extraction mode, extraction time are in their high level (+1) and the variables ratio, morphotype and variety in their low level (-1) (experiment 6).

Bioactive compounds contents are ranged between 28 ± 1 to 350 ± 13 mg/L GAE, 138 ± 1 to 1653 ± 36 mg/L QE and 33 ± 1 to 365 ± 2 mg/L CE for TPC, TFC and CTC, respectively (Table 5). Total polyphenol, Total flavonoid and condensed tannin yields were influenced by the variables extraction solution (S), ratio (w/v) (R), extraction mode (M) and variety (V).

Table 6 illustrates estimation and statistics of linear regression coefficient. Coefficient is known as statistically significant if its absolute value is strictly higher than the double of the experimental standard deviation, $|\text{coef}| > 2\sigma$ [39]. The determination of the each coefficient and experimental standard deviations were determined by the method of linear regression [40].

Table 5. Total polyphenol, total flavonoid and condensed tannin content of kola nuts samples according to Plackett-Burman design

Test set	Independent variables						Experimental responses		
	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	Y ₁ ^a	Y ₂ ^b	Y ₃ ^c
1	+	+	+	-	+	-	234±3	777±11	161±1
2	-	+	+	+	-	+	147±5	680±7	205±1
3	-	-	+	+	+	-	113±4**	600±13**	180±1
4	+	-	-	+	+	+	227±9*	1193±22	238±2
5	-	+	-	-	+	+	28±1	138±1	33±1
6	+	-	+	-	-	+	350±13	1653±36	365±2
7	+	+	-	+	-	-	283±2	1157±4	285±1
8	-	-	-	-	-	-	110±7**	617±18**	148±1

Data of the same column having the same sign are statistically in the same homogenous group at P=.05

Y₁: Total polyphenol content; Y₂: Total flavonoid content; Y₃: Condensed tannin content

a: mg/L GAE; b: mg/L QE; c: mg/L CE

Table 6. Statistical estimates of coefficient and standard deviation

Coefficient	Coefficient and standard deviations for each equation					
	Total polyphenols		Total flavonoids		Condensed tannins	
	Values	2σ	Values	2σ	Values	2σ
a ₀	186.5*		843.5*		201.94*	
a ₁	86.83*		334.83*		60.41*	
a ₂	-13.5		-155.67*		-30.86*	
a ₃	24.66*	14	67.33*	53.66	25.79	27.67
a ₄	6		64*		25.12	
a ₅	-36*		-166.5*		-49.06*	
a ₆	1.5		56*		8.40	

*:significant data at P=.05

Statistical analysis showed that extraction solvent, extraction process and variety had the greatest effect on total phenolics extraction. The main variable for condensed tannins extraction were extraction solvent, solid-liquid ratio (w/v) and variety. However, for total flavonoids extraction, all variables were dominating. The levels of the non-dominating factors were fixed in order to allow the optimization.

The factors X₁ (extraction solution), X₂ (solid-liquid ratio), X₃ (extraction mode) and X₅ (variety) have been selected as the most influential in kola nuts compounds extraction according to the effect estimated values (coefficients of the equation). The new experiment was conducted by setting the non-dominating factors "morphotype" (X₄) and "extraction time" (X₆) to their high level (+1), respectively M=Red and T (h)=24.

Minimal effect of the morphotype will make it possible to choose indifferently any cultivar for the future analysis.

3.3 Optimization of Phenolics Compounds Extraction

The full factorial design used was determined the combination of different levels of influential parameters that give the best compounds yields. TFC, TFC and CTC were determined. For that 16 experiments (2⁴) were conducted according to the matrix presented in Table 7.

The values of regression coefficient determined are given in Table 8. The effect of individual variables and interactions effects was estimated [41].

Table 8 shows that all variables presented significant effect on total phenolics extraction. The most important parameter affecting this extraction is the solvent. The interaction between solvent (X₁) and ratio (X₂) is significant. The predictive equation of total phenolics yields (Y₁), neglecting the non-significant factors, is given by equation 1 with a satisfactory R² value (R²=0.98).

$$Y_1 = 230.21 + 49.29X_1 - 51.04X_2 + 14.95X_3 - 31.37X_5 + 18.87X_1X_2 \quad (1)$$

The higher total phenolics contents (358 mg/L GAE) was obtained when variables extraction solvent and extraction mode are in their high level (+1) and variables ratio and variety in their low level (-1).

Total flavonoids extraction was affected by solvent (X_1), ratio (X_2) and variety (X_5). No interaction between factors are noted. The most important parameter affecting this extraction is the solvent (Table 8). The data showed a good fit with equation 2, being were statistically acceptable at $P=0.05$ level and adequate with a satisfactory R^2 value ($R^2=0.97$). Equation 2 being developed to present the relationships between TFC and extraction variables.

$$Y_2=854.08+325.92X_1-119.25X_2-149.58X_5 \quad (2)$$

The highest value of total flavonoid (1449 mg/L QE) was obtained when the variable extraction solvent is in his high level (+1) and variables ratio and variety in their low level (-1).

Condensed tannin extraction was affected by by all variables. The most important parameter affecting tannin extraction is the same as in the case of total phenolics extraction (factor X_1). Two significant interactions were observed: solvent (X_1) - ratio (X_2) and solvent (X_1) - variety (X_5). Equation 3 describe the model of condensed tannin with a satisfactory R^2 value ($R^2=0.96$).

$$Y_3=159.85+58.84X_1+22.73X_2+21.06X_3-20.53X_5+23.24X_1X_2+15.62X_1X_5 \quad (3)$$

The highest value of condensed tannin (267.39 mg/L CE) was obtained also when variables solvent, ratio, extraction mode are in their high level and variable variety in their low level.

The experimental analysis showed that polyphenolics compounds extraction of kola nuts is favored when the variables extraction solvent and mode are in their high level (+1), and variables ratio and variety in their low level (-1). Thus, the optimum extraction process of kola nuts bioactive compounds involves the following parameters:

- Extraction solvent : Aqueous ethanol (50%)

Selecting the right solvent affects the amount and rate of polyphenols extracted [42]. The use of water with a organic solvent, for the extraction of polyphenols from some plant materials has been reported to contribute to the creation of a moderately polar medium that insures the extraction of phenolics, giving better results

than when using a pure organic solvent [12,13]. According to Mokhtarpour et al. [15], it was suggested the using of 50% aqueous ethanol for plant tannin extraction. Ethanol is a good solvent for polyphenol extraction and is safe for human consumption [43].

- Ratio (w/v) : 1/100

A correct ratio of solvent and plant matter is fundamental for obtaining an optimal extraction process. We note an increasing of TPC, TFC and CTC when extraction solution volume move from 20 mL (5/100) to 100 mL (1/100). According to Sampath [10], when the solvent volume was increased, it can increase the absorption rate, swelling rate and diffusion rate of the plant cell wall. At the same time, excessive solvent volume, promotes the extraction of undesired compound from the plant material, affect the quality of desired compounds and decrease the yield also. This decrease due to the fact that when the ratio reached a certain level, the extract may be well saturated [44,45,38].

- Extraction mode : infusion

The temperature increase would favor the diffusion of the compounds of the vegetable matrix to the extraction solution [12]. In this study, heating the extraction solvent promotes the diffusion of the sample compounds, kola nuts bioactive compounds would thermostable. Temperature's effect on extraction is dual. On one hand, higher temperature can accelerate the solvent flow and thus increase the content and on the other hand, higher temperature can decrease the fluid density that may reduce the extraction efficiency [45].

- Variety : traditional

Genetic modification of kola nut could explain the higher efficiency of traditional nuts compared to improved nuts. A similar report by Nyamien et al. [38], revealed that kola nuts caffeine content depends on the type of variety used.

3.4 Validation of 2⁴ Full Factorial Design Optimization of Phenolics Compounds from Kola Nuts

All the models were established with high coefficient of determination R^2 , ranging from 0.96 to 0.98, wich means a close agreement between the experimental results and those predicted by the models. The predictive quality of every model was also tested at the recommended optimum

condition. All the responses were replicated three times at the optimum condition, and the results are presented in Table 9. The arithmetic means of the experimental values was 350±11 mg/L GAE, 1460±9 mg/L QE and 264.33±2 mg/L CE for TPC, TFC and CTC, respectively.

Table 7. Experimental design (2^k, k=4) and corresponding responses

Run order	Technological parameters and coded levels				Responses		
	X ₁	X ₂	X ₃	X ₅	Y ₁ ^a	Y ₂ ^b	Y ₃ ^c
1	W	1/100	M	T	247±18 ^{**}	727±4	120±1
2	E	1/100	M	T	347±4 ⁺	1587±16	203±1
3	W	5/100	M	T	119±4	459±11 ⁺	97±1 ^{**}
4	E	5/100	M	T	288±13 ^{***}	1253±11	282±1
5	W	1/100	I	T	313±9	933±4	159±1
6	E	1/100	I	T	353±9 ⁺	1373±4	190±1
7	W	5/100	I	T	167±22	571±9	173±7
8	E	5/100	I	T	259±31 ^{**}	1127±4 ^{**}	241±1
9	W	1/100	M	lp	193±11 ^{**}	440±1 ⁺	30±1 ⁺
10	E	1/100	M	lp	263±11 ^{**}	1013±16	139±1
11	W	5/100	M	lp	67±9	187±9	31±1 ⁺
12	E	5/100	M	lp	197±4 ⁺	873±9	214±1
13	W	1/100	I	lp	250±1 ^{**}	620±1	97±1 ^{**}
14	E	1/100	I	lp	283±11 ^{***}	1093±4 ^{**}	138±1
15	W	5/100	I	lp	91±4	289±11	100±1
16	E	5/100	I	lp	245±11 ^{**}	1120±13 ^{**}	327±1

Data of the same column having the same sign are statistically in the same homogenous group at P=.05
W: water ; E: ethanol 50% ; I: infusion ; M: maceration ; T: traditional ; lp: improve a: mg/L GAE; b: mg/L QE;
c: mg/L CE

Table 8. Statistical estimates of coefficient and standard deviation of 2⁴ full factorial experiments

Coef	Model coefficients					
	Total polyphenols		Total flavonoids		Condensed tannins	
	Values	2σ	Values	2σ	Values	2σ
b ₀	230.21		854.08		159.45	
b ₁	49.29 ⁺		325.92 ⁺		58.84 ⁺	
b ₂	-51.04 [*]		-119.25 ⁺		22.73 ⁺	
b ₃	14.95 ⁺		36.75 ⁺		21.06 ⁺	
b ₅	-31.37 [*]		-149.58 ⁺		-20.53 ⁺	
b ₁₂	18.87 ⁺	9.66	32.58	51.98	23.24 ⁺	13.05
b ₁₃	-9.29		-38.41		-10.52	
b ₁₅	-0.79		-5.41		15.62	
b ₂₃	-3.79		5.08		6.8	
b ₂₅	2.37		32.08		10.26	
b ₃₅	3.54		39.42		10.58	

*: significant data, Coefficient is known as statistically significant if its absolute value is strictly higher than the double of the experimental standard deviation, |coef|>2σ [39]

Table 9. Experimental data for verification of the models predicted at optimal condition

Optimal condition	Response 1		Response 2		Response 3	
	TPC (mg/L GAE)		TFC (mg/L QE)		CTC (mg/L CE)	
	Pred	Exp	Pred	Exp	Pred	Exp
X ₁ =Ethanol 50%						
X ₂ = Ratio 1/100						
X ₃ = Infusion	358 [*]	350±11 [*]	1449 ^{**}	1460±9 ^{**}	267.39 ^{***}	264.33±2 ^{***}
X ₄ = Traditional						

Data of the same column having the same sign are statistically in the same homogenous group at P=.05
Pred= predicted values, Exp= experimental values

Experimented data were approaching the predicted values. This indicated that the optimization achieved in the present study was reliable. Deviations between experimental values and the predicted values can be explained by the lack of perfectly fitted models and experimental errors.

4. CONCLUSION

Kola nuts occupy a unique place amongst West Africans where these are widely consumed. These are the important source of bioactive compounds, and can be used as a possible source of natural antioxidants for African's population and European industries. The use of experimental design in this study showed the optimum conditions for extracting bioactive compound from kola nuts by non toxic solvent (aqueous ethanol in particular). All the developed models showed an adequate predictive quality and the optimal condition was predicted by desirability function and successively verified. The optimum conditions for obtaining high yield of polyphenols content were extraction by infusion of traditional variety at solid-liquid ratio 1/100 with aqueous ethanol 50%. A meticulous study of each factor must be done to obtain more significant yields of kola nuts compounds. The researchers may determine precise phenolics contents of the most widely eaten kola to avoid consumption in high dose since our knowledge on risks is much limited. Extraction with non toxic solvent may allow the isolation of directly edible ingredients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Momo C, Ngwa A, Dongmo G, Oben J. Antioxidant properties and alpha-amylase inhibition of *Terminalia superba*, *Albizia* sp., *Cola nitida*, *Cola odorata* and *Harungana madagascarensis* used in the management of diabetes in Cameroon. *J. Health Sci.* 2009;55(5):732-738.
- Laib I and Barkat M. Composition chimique et activité antioxydante de l'huile essentielle des fleurs sèches de *Lavandula officinalis*. *Agric.* 2011;2:89-101. French
- Fernande N, Marthe T, Laure N, Enyong J. *In vitro* antioxidant activity of *Guibourtia tessmannii* Harms, J. Leonard (Cesalpinoideae). *J. Med. Plant Res.* 2013; 7(42):3081-3088.
- Mosquera O, Correa Y, Buitrago D, Nino J. Antioxidant activity of twenty five plants from Colombian biodiversity. *Mem Inst Oswaldo Cruz.* 2007;102(5):631-634.
- Scalbert A, Manach C, Morand C, Rémésy C. Dietary polyphenols and the prevention of diseases. *Crit Rev Food Sci Nutr.* 2005; 45:287-306.
- Omar A. The antioxidant activity and polyphenolic contents of different plant seeds extracts. *Pak. J. Biol. Sci.* 2009; 12(15):1063-1068.
- Santos D, Cavalcanti R, Rostagno M, Queiroga C, Eberlin M, Meireles A. Extraction of polyphenols and anthocyanins from the Jambul (*Syzygium cumini*) fruits peels. *Food Public Health.* 2013;3(1):12-20.
- Dai J, Mumper R. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules.* 2010;15:7313-7352.
- Tatiya A, Tapadiya G, Kotecha S, Surana S. Effect of solvents on total phenolics, antioxidant and antimicrobial properties of *Bridelia retusa* Spreng. stem bark. *Indian J. Nat. Prod. Resour.* 2011;2(4):442-447.
- Sampath M. Optimization of the extraction process of phenolic antioxidant from *Polyalthia longifolia* (Sonn.) Thawaites. *J. Appl. Pharm. Sci.* 2013;3(2):148-152.
- Cheng A, Yan H, Han C, Chen X, Wang W, Xie C, et al. Acid and alkaline hydrolysis extraction of non-extractable polyphenols in blueberries: optimisation by response surface methodology. *Czech J. Food Sci.* 2014;32(3):218-225.
- Shi J, Yu J, Pohorly J, Young C, Bryan M, Wu Y. Optimization of the extraction of polyphenols from grape seed meal by aqueous ethanol solution. *Food Agric. Environ.* 2003;1(2):42-47.
- Mussatto S, Ballesteros L, Martins S, Teixeira J. Extraction of antioxidant phenolic compounds from spent coffee grounds. *Sep. Purif. Technol.* 2011;83:173-179.
- Selvaraj K, Chowdhury R, Bhattacharjee C. Optimization of the solvent extraction of bioactive polyphenolic compounds from aquatic fern *Azolla microphylla* using response surface methodology. *Int. Food Res. J.* 2014;21(4):1559-1567.

15. Mokhtarpour A, Naserian A, Valizadeh R, Mesgaran D, Pourmollae F. Extraction of phenolic compounds and tannins from Pistachio by-products. *Annu. Res. Rev. Biol.* 2014;4(8):1330-1338.
16. Koffi E, Sea T, Dodeye Y, Soro S. Effect of solvent type on extraction of polyphenols from twenty three Ivorian plants. *Journal of Animal and Plant Sciences.* 2010;5(3):550-558.
17. Arhewoh M, Falodun A, Okhamade A, Boa Y, Sheng Q. Ultrasonic assisted extraction and radical scavenging activity of some selected medicinal plants. *J. Pharm. Res.* 2011;4(2):408-410.
18. Anurukvorakun O. Factorial design applied to subcritical water extraction for the investigation of flavonoids and antioxidant capacity of *Gynura calciphila* Kerr. Mahidol University J. *Pharma. Sci.* 2013;40(2):7-16.
19. Nyamien Y, Adjé F, Niamké F, Chatigre O, Adima A, Biego H. Caffeine and phenolic compounds in *Cola nitida* (Vent.) Schott and Endl and *Garcinia kola* Heckel grown in Côte d'Ivoire. *Br. J. Appl. Sci. Tech.* 2014;4(35):4846-4859.
20. Kuzma P, Druzynska B, Obiedzinski M. Optimization of extraction conditions of some polyphenolic compounds from parsley leaves (*Petroselinum crispum*). *Acta Sci. Pol., Technol. Aliment.* 2014; 13(2):145-154.
21. Niemenak N, Onomo P, Fotso, Lieberei R, Ndoumou D. Purine alkaloids and phenolic compounds in three *Cola* species and *Garcinia kola* grown in Cameroon. *S. Afr. J. Bot.* 2008;74:629-638.
22. Boudjeko T, Rihouey C, Ndoumou D, El Hadrami I. Characterisation of cell wall polysaccharides, arabinogalactans-proteins (AGPs) and phenolics of *Cola nitida*, *Cola acuminata* and *Garcinia kola* seeds. *Carbohydr. Polym.* 2009;78:820-827.
23. Tende A, Ezekiel I, Dare S, Okpanachi O, Kemuma O, Goji T. Study of the effect of aqueous extract of kolanut (*Cola nitida*) on gastric acid secretion and ulcer in the white wistar rats. *Br. J. Pharmacol. Toxicol.* 2011;2(3):132-134.
24. Aikpokpodion P, Oduwale O, Adebisi S. Appraisal of pesticide residues in kola nuts obtained from selected markets in Southwestern, Nigeria. *J. Sci. Res. Rep.* 2013;2(2):582-597.
25. Prohp T, Ekpo K, Osagie E, Osagie A, Obi H. Polyphenol contents and polyphenol oxidase activities of some Nigerian kolanuts. *Pak. J. Nutr.* 2009;8(7):1030-1031.
26. Okoli J, Abdullahi K, Myina O, Iwu G. Caffeine content of three Nigerian cola. *J. Emerg. Trends Eng. Appl. Sci.* 2012;3(5): 830-833.
27. Arhewoh M, Falodun A, Okhamade A, Boa Y, Sheng Q. Ultrasonic assisted extraction and radical scavenging activity of some selected medicinal plants. *J. Pharm. Res.* 2011;4(2):408-410.
28. Umoren E, Osimand E, Udoh P. The comparative effects of chronic consumption of kola nut (*Cola nitida*) and caffeine diets on locomotor behaviour and body weights in mice. *Niger. J. Physiol. Sci.* 2009;24(1):73-78.
29. Rajendran A, Thirugnanam M, Thangavelu V. Statistical evaluation of medium components by Plackett-Burmann experimental design and kinetic modeling of lipase production by *Pseudomonas fluorescens*. *Indian J. Biotechnol.* 2007; 6:469-478.
30. Analytical Methods Committee, AMCTB N°55. Experimental design and optimization (4): Plackett-Burman designs. *Anal. Methods.* 2013;5:1901-1903.
31. Wahidu Z. Optimization of antioxidant extraction from jackfruit (*Artocarpus heterophyllus* Lam.) seeds using response surface methodology. Master of Science in Nutrition and Natural Development, Faculty of Bioscience Engineering: Ghent University; 2012.
32. Aboua N, Yao B, Gueu S, Trokourey A. Optimization by experimental design of activated carbons preparation and their use for lead and chromium ions sorption. *Res. J. Agric. Biol. Sci.* 2010;6(6):665-670.
33. Mylonaki S, Kiassos E, Makris D, Kefalas P. Optimization of the extraction of olive (*Olea europea*) leaf phenolics using water/ethanol based solvent system and response surface methodology, *Anal. Bioanal. Chem.* 2008;392(5):977-985.
34. Singleton V, Rossi J. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Viticult.* 1965; 16:144-158.
35. Koffi E, Le Guernevé C, Lozano P, Meudec E, Adjé F, Bekro Y et al. Polyphenol extraction and characterization of *Justicia secunda* Vahl leaves for traditional medicinal uses. *Ind Crpos Prod.* 2013;49:682-689.

36. Marinova D, Ribarova F, Atanassova M. Total phenolics in bulgarian fruits and vegetables. J. Univ. Chem. Technol. Metal. 2005;40(3):255-260.
37. Heimler D, Vignolini P, Din M, Vinueri F, Ronani A. Antiradical activity and polyphenol composition of local Brassicaceae edible varieties. Food Chem. 2006;99(3):464-469.
38. Mouchine F, Abdellah F, Bouchaib I, Taoufik H, Saad R. The application of Plackett and Burman design in screening the parameters acting on the hydrodistillation process of Moroccan rosemary (*Rosmarinus officinalis* L.). Int. J. Innov. Appl. Stud. 2014;8(1):372-381.
39. Nyamien Y, Adjé F, Niamké F, Koffi E, Chatigre O, Adima A, et al. Effect of solvents and solid-liquid ratio on caffeine extraction from Côte d'Ivoire kola nuts (*Cola nitida*). Int. J. Sci. Res. 2015;4(1): 218-222.
40. Assidjo E, Yao B, Akou E, Ado G. Optimisation of the treatment conditions of cocoa butter in order to reduce non-quality. Journal of chemom. 2005;19:543-548.
41. Feinberg M. La validation des méthodes d'analyse: Approche chimométrique de l'assurance qualité au laboratoire, Paris:Masson; 1996. French.
42. Nese Ö, Duygu K. Boron removal from aqueous solutions by batch absorption onto cerium oxide using full factorial design. Desalination. 2008;223(1):106-112.
43. Xu B and Chang S. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. J Food Sci. 2007; 72(2):159-166.
44. Xi J. Caffeine extraction from green tea leaves assisted by high pressure processing. J. Food Eng. 2009;94:105-109.
45. Sathishkumar T, Shanmugam S, Rajasaekaran P, Sadavivam S, Manikandan V. Optimization of flavonoids extraction from the leaves of *Tabernaemontana heynena* Wall. using L₁₆ orthogonal design. Nat. Sci. 2008;6(3):10-21.

© 2015 Nyamien et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sciencedomain.org/review-history.php?iid=1041&id=11&aid=8646>