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The Impact of Post-Harvest Traditional Technologies on Nutritional Value and Antioxidant Activity of Seeds Kernels "akpi" of Côte d'Ivoire (West Africa)

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Abstract Proximate composition (moisture, protein, total dietary fibre, lipids, ash, phenolic compounds, acidity and pH), minerals (K, Ca, Mg, P, Cl and K) and trace elements (Fe, Zn) were determined in *Ricinodendron heudelotii* (Baill.) Pierre ex Pax seeds kernels found in six producing areas of Côte d'Ivoire (West Africa). There were significant differences between the *Ricinodendron heudelotii* seeds kernels samples for all the parameters studied; in addition, both the locality of production and the *Ricinodendron heudelotii* seeds kernels extraction systems showed a noteworthy influence on the mineral and element composition of the seeds kernels. The seeds kernels from *Ricinodendron heudelotii* were rich in lipids (47.8-54.8%), protein (23.4-28.3%) and energy (159.2-267.0 kcal/100g). Phosphorus (1.8-2.3%) was the predominant mineral. Polyphenols (54.56-156.8 mg/100g) and oxalates (382.6-593.3 mg/100g) were the main phytochemical compounds identified. It is worth mentioning that, the consumption of seeds kernels may contribute relatively high intake levels of lipids, protein, phenolic compounds, mineral and micronutrient (K, Mg, Ca, Mn, Fe and Zn). Application of multidimensional scaling (MDS) correctly classified the seeds kernels from *Ricinodendron heudelotii* according to the kernels extraction into four extraction system: Agboville1-Bondoukou diagram, Agboville2-Vavoua diagram, Divo diagram and Lakota diagram.

Keywords *Ricinodendron heudelotii*, Traditional extraction method, Nutritive value, Antioxidant properties, Seeds kernels, Multidimensional scaling

1. Introduction

In Africa and Cote d'Ivoire (West Africa) in particular, several wild fruits and vegetables are consumed among the species of non-timber forest products (NTFPs) [1]. These wild fruits and vegetable contribute significantly to meeting rural nutritional needs and a source of household income [2]. Notable among these wild fruits and vegetables is the *Ricinodendron heudelotii* (Baill. Pierre ex Pax) a member of the *Euphorbiaceae* family. The plant is known to have two subspecies, namely, *heudelotii* and *africanum* (Müll. Arg.) [3]. Subspecies *heudelotii* is known to be common in Senegal and Benin, while the subspecies *africanum* is predominant in the southern part of Nigeria and South Africa. The subspecies *R. heudelotti* is the only one found in Côte d'Ivoire. *Ricinodendron heudelotii* (*R. heudelotti*) specie is a fast-growing tree, reaching up to 50 m in height and 2.7 m in girth or a diameter of 150 cm, which grows in the world's tropical area. In Sub-Saharan Africa, it is one of the main trees of the tropical forest in the equatorial region. The tree has several local names such as "akpi" in Côte d'Ivoire, "Njansang" in Cameroon, "Okwe" in south east Nigeria, "Bomoko" in Central Africa Republic, and "Betratra" in Madagascar [4]. The edible part of the fruit (kernel) is rich in lipids (47.8 - 50%) but also contains proteins (22.38 - 24.91%), phosphorus (1693 mg/100 g), calcium (1013 mg/100 g), potassium (811.4 mg/100 g) and magnesium (528.6 mg/100 g) [4, 5]. Several kernel extraction technologies exist independently of production areas. Kernels from *R. heudelotii* fruits are extracted depending on the capacity of the women. Generally, drupes that fall-down from the trees are piled and left to ferment for two weeks or more to enable the pulp to rot. Once rotten, the seeds can be extracted by washing and boiling, and are then put into cold water and left for 24 hours. They are then subjected to further boiling, which cracks the seed shells exposing the kernels for extraction by knife or any other

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sharp object. The kernels are then dried in the sun or in an oven. Once dried, these kernels can be stored for several years and may be sold throughout the year in urban markets [6]. The seeds of «Akpi» (*Ricinodendron heudelotii*), because of its very pronounced and appreciated aroma, are extensively used in many diets in Africa, more specifically in Côte D'Ivoire. Usually, the kernels of the plant are used in African soups as an additive function (e.g. as a thickener) and taste lifter [7]. Studies on post-harvest and processing technologies for fruits, nuts and kernels have shown that, the chemical and nutritional property are one of the factors of food variation that constitutes the kernel extraction systems [8]. Despite the increasing number of scientific reports on the *R. heudelotti* [9-11], studies on the overall nutritional quality of seeds kernels from Côte d'Ivoire are few. With the aim of defatted producing defatted flours, knowledge of its nutrients and some anti-nutrients composition with respect to traditional extraction process need to be generated. The objectives of this study therefore, was to investigate the nutrients, minerals, phytochemical and bioavailability of flours from *R. heudelotti* (Baill.) Pierre ex Pax seeds kernels found in six producing areas of Côte d'Ivoire. In this study, the effect of traditional kernels extraction process on the physico-chemical and antioxidant properties of kernels flours from *R. heudelotti* was assessed.

2. Materials and Methods

2.1. Materials Chemicals and Reagents

All chemicals and reagents used were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ultra-pure water with a resistivity of 18.2 MΩ was used in all experiments provided by ELGA (Flex III, U.K) water purification system.

2.2. Sampling and Sample Preparation

The plant material was mainly the kernels of *R. heudilotii* (Figure 1).

Thus, fresh *R. heudilotii* kernels were collected from six different producing areas of Côte d'Ivoire (West Africa): Bondoukou, Agboville1, Agboville2, Divo, Lakota and Vavoua. The unit operations involved in each methods of kernels extraction processes are shown in figure 2. After collection, samples (n = 6) were immediately transferred to the Food Biochemical and Tropical Products Technology Laboratory (Abidjan, Côte d'Ivoire) for preparation of the defatted flour. Upon arrival, the kernels were cleaned manually to remove all foreign materials and dried in an oven at 40°C for 24 h prior to analysis. The dried material was grinded into fine flour with a laboratory blender (Bimby mod. 2200, Vorwerk, Wuppertal, Germany). All the analyses were done, in triplicate on flour obtained by pooling all the samples in the same amount.

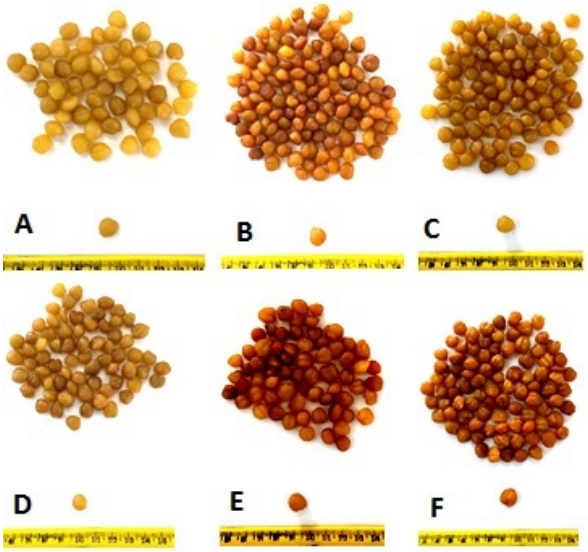


Figure 1. *R. heudelotii* seeds kernels samples "Bondoukou" (A), "Agboville2", (B), "Divo" (C), "Lakota" (D), "Vavoua " (E) and "Agboville1" (F)

2.3. Proximate Analysis

The methods used for sample treatment and analysis (Moisture, ash, lipids, protein) were carried out following standard procedures recommended by AOAC [12]. Moisture was determined by gravimetric method, heating in an oven at 105±1°C until constant mass. Total nitrogen was determined by the Kjeldahl method and converted into protein, using factor 6.25. Total lipids were extracted by the Soxhlet technique with hot solvent (hexan) and afterwards were determined by gravimetric. Ash was determined by gravimetric of incinerated sample, in muffle, at 550°C. The total carbohydrates (TCHO) were calculated by difference as suggested by FAO/WHO procedure [13]. The total dietary fiber content was determined following Prosky's protocol [14]. Total sugars (TS) were determined using the by the phenol-sulphuric acid method [15] while the reducing sugars were quantified by the oxido-reduction method using 3.5-dinitrosalysislic acid (DNS) as oxidizing agent [16]. A reaction mixture was prepared by adding 0.1 ml of sample, 0.9 ml of ultra-pure water and 0.5 ml of DNS. The mixture was heated to the water-bath 100°C for 5 min and then cooled down to room temperature and diluted with distilled water to 3.5 mL. After rapid cooling during 30 min, the absorbance was recorded at 480 nm. As for total sugars, 0.1 ml from the sample was added to 0.9 ml distilled water and then 1 ml phenol [5% (p/v)] and 1 ml H_2SO_4 extract were added. After the softly homogenized, the mixture was heated to the water-bath 100°C for 15 min. The absorbance was measured at 540 nm. The energy content of the sample was computed from the proximate data using the Atwater formula. It was estimated by multiplying each gram of carbohydrate, protein and lipid by 4 kcal, 4 kcal and 9 kcal respectively [17]. All analyses were performed in triplicate.

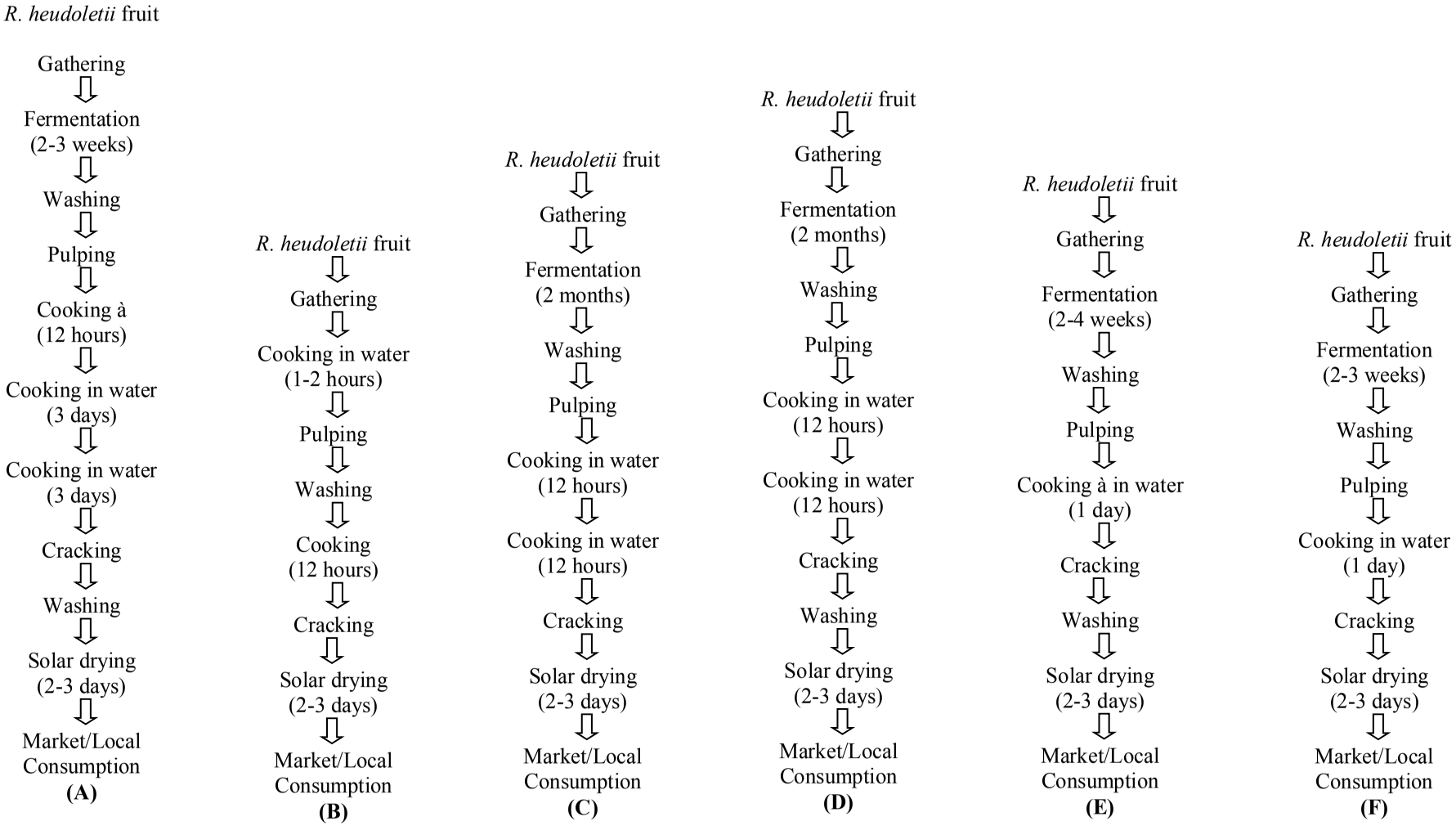


Figure 2. Flow diagram of the process of harvesting of seeds and kernels extraction from *R. heudelotii* seed from six producing areas of Côte d'Ivoire: Bondoukou (A); Agboville2 (B); Divo (C); Lakota (D); Vavoua (E); Agboville1 (F)

2.4. Mineral Composition

The sample was accurately weighted and dry-ashed (550°C, one night; method 40–70.01) [18] in a muffle furnace (Cavallo Srl, Buccinasco, Italy). Grey ashes were treated with high purity hydrogen peroxide (H_2O_2 30% Suprapur, St. Louis, MO, USA) to obtain white ashes, that were dissolved with acid solution (2 mL HCl 30% Suprapur, St. Louis, MO, USA) and diluted with distilled water in volumetric flasks. Mineral concentrations (potassium (K), magnesium (Mg), iron (Fe), calcium (Ca) and zinc (Zn)) were determined by Atomic Absorption (Analyst 800 Perkin Elmer, Waltham, MA, USA), while phosphorous (P) - and Chlorine (Cl) were determined by a colorimetric method using Cary 3E UV-VIS Spectrophotometer (Varian, Mulgrave, Australia) [19]. All analyses were carried out in triplicate with two measurements per analysis. All minerals were reported as mean and standard deviation; data are expressed as% dry weight.

2.5. pH Determination

Ten grams of powdered sample was diluted with 90 ml distilled water and mixed thoroughly and the pH of the sample was measured using a pH meter model 744 (Metrohm AG, Herisau, Switzerland) according to the procedure described in the Swiss Food Manual (Schweizerisches Lebensmittel-Buch, 2001). The pH meter was calibrated with standard buffers 4 and 7, before measuring the pH of the mixture.

2.6. Total Titrable Acidity (TTA)

The acidity of kernels was determined by titration following the method described by AOAC [12] using phenolphthalein (Sigma–Aldrich Chemical Co., St. Louis, MO, USA) as an indicator. The acidity of the samples was calculated by using the following equation

$$\text{Titrable acidity (\%)} = 0.0090 \times \text{volume of NaOH used} /100 \quad (1)$$

2.7. Phytochemical Analysis

2.7.1. Total Flavonoids Determination

Total flavonoid content was determined using a colorimetric assay as described by the method of Meda *et al.* [20]. Sample extracts were evaporated to dryness and re-dissolved in 80% ethanol to be ready for the analytical test. Briefly, 1 mL of a sample (ethanolic solutions or *R. heudilotii* kernels extract) was mixed with 3 mL 95% ethanol (v/v), 0.2 mL 10% aluminum chloride (m/V), 0.2 mL of 1 mol/L potassium acetate and 5.6 mL water. A volume of 10% (m/V) aluminum chloride was substituted by the same volume of distilled water and used as a blank. After incubation at room temperature for 30 minutes, the absorbance was measured at 415 nm using a UVVIS spectrophotometer (Cary 50 Bio, Varian Australia Pty. Ltd., Victoria, Australia). Quercetin (Sigma, St. Louis, MO, USA) was used to perform the calibration curve (standard solutions of 6.25, 12.5, 25.0, 50.0, 80.0 and 100.0 µg/mL) in 80% ethanol (V/V). Flavonoids in *R. heudilotii* seeds kernels extracts were expressed as mg quercetin equivalents per gram of dried sample (mg QE/g).

2.7.2. Phytic Acid Content

Phytic acid was determined by the New Chromophore method of Mohammed *et al.* [21]. Briefly, a mass of 0.5 g of the sample is homogenized in 25 mL of 3% trichloroacetic acid (TCA) for 45 min and then centrifuged at 3500 rpm for 15 min. To five (5) mL of the supernatant obtained are added 3 mL of 1% iron chloride prepared in hydrochloric acid HCl (1 N) and then heated on a water bath for 45 min. After cooling the mixture, 5 mL of hydrochloric acid (HCl) are added and the mixture is then left to stand for 2 hours. Five (5) mL of 1.5 N sodium hydroxide are then added to the mixture obtained above; the whole is carried on a water bath for 15 min and centrifuged again at 3500 rpm for 15 min after cooling. One milliliter of the supernatant is removed, to which is added 4.5 mL of distilled water, 4.5 mL of ortho-phenanthroline reagent. The optical density is read at 470 nm on the spectrophotometer against a blank. The amount of phytate content were expressed as mg/100 g dry weight.

2.7.3. Tannins

Tannins were determined using acidified vanillin and (+) catechin as standard [22]. To determine tannins in *R. heudelotti* seeds kernels two grams of sample were mixed with 30 mL acetone 80%. The mixture was stirred for 15 min and filtered under pressure. Acetone was separated using a rotavapor (Buchi, R124) and then mixed with freshly prepared 4% vanillin in ethanol. The mixture were stirred, treated with concentrated HCl and quantified by spectrophotometry (PG Instruments, England) at 500 nm. Tannins content of samples was estimated using a calibration curve for (+)-catechin. The results obtained were expressed as mg catechin equivalent/g of sample, on a dry weight basis.

2.7.4. Oxalates

Oxalates content was determined by using the method described by Day and Underwood [23]. Briefly, one gram of dried powdered was weighed into 100 mL conical flask. 75 mL of 1.5 N H_2SO_4 was added and the solution was carefully stirred intermittently with a magnetic stirrer for about 1 h and then filtered using Whatman no1 filter paper. 25 mL of sample extract (filtrate) was collected and titrated hot (80-90°C) against 0.1 N $KMnO_4$ solution to the point when a faint pink colour appeared that persisted for at least 30 sec. The results were expressed as mg/100 g dry weight.

2.7.5. Total Phenolic Assay

The phenolic compounds were extracted following the procedure described by N'Dri *et al*. [24], and determined by the Folin-Ciocalteu assay [25]. Briefly, a dried sample (1 g)

was extracted with 10 mL of 80% methanol at room temperature and then reacted with a 10-fold diluted Folin-Ciocalteau reagent. Sodium carbonate at a concentration of 6% (w/v) was added, and the final volume was made up with deionized water. After incubation at room temperature for 15 min, the mixture's absorbance was measured against the gallic acid standard (the blank being prepared under the same conditions as before but without extract) at 725 nm using a spectrophotometer (PG Instruments, England). Total phenolic content was expressed as mg gallic acid equivalent (GAE)/100 g sample.

2.8. Statistical Analyses

The data obtained were subjected to statistical analysis performed with Statistica 9.0 (StatSoft, Krakow, Poland). They were recorded as means ± standard deviation (SD), and analysed by Excel 2013. Analysis of variance (ANOVA) were used to study the differences between samples. Duncan's multiple range test ($p < .05$) was used to determine the significances within treatments. The similarity between extraction systems of the seeds kernels were examined by multidimensional scaling (MDS). A proximity matrix was required to perform multidimensional scaling (MDS), which is a collection of similarity estimates between each pair of samples in the set of chemical constituents. Proximity was determined by simple Euclidean distance applied to standardized data. Similarity groups were compared by means of one-way ANOVA test with Duncan's multiple range post hoc, assuming there were significant differences among them when the statistical comparison gave $P < 0.05$. All analyses were performed in duplicate or triplicate.

3. Results and Discussion

In this study, the effect of kernels extraction methods on chemical composition and antioxidant activity of flours from *R. heudelotti* kernels were assessed.

Firstly, we performed a nutritional characterization of kernels samples from *R. heudelotii* differentiating them according to the site of production. The findings were presented in Table 1. Overall, there were significant differences ($P < 0.05$) between the kernels samples for all the parameters studied. A difference that could be attributed to its steps of preparation (post-harvest traditional technologies) and to the environmental condition. Proximate composition confirm that the seeds kernels from *R. heudelotii* has potential high lipid and protein content to satisfy calorie and the protein demand of the populations. They contain considerable amounts of proteins (23.4-28.3%), higher than protein rich foods such as quinoa [26], Bambara groundnut [27], cowpeas [28] seeds which ranged between 13.5 - 26.8%. Nevertheless, lipids are a major component of kernels (47.8% - 54.8%). These finding were in agreement with other studies which reported that the lipids content can vary from 47.0 to 60.0% [29, 30]. The high oil content suggests that *R. heudelotii* can be used as potential source of raw material for commercial activities such as for *Millettia ferruginea* seed [31]. Furthermore, intrinsic differences between proteins on the one hand and lipids on the other hand (Table 1) may be explained by the compositional changes following the degradation of other constituents during the cooking steps [32]. For the vegetables and legumes for example, pre-cooking step in the production process appears to influence the protein content. Heating before cooking improves the nutritional value and the availability of nutrients [33], which could be the case in this study. The fact that the kernels from Divo's production process came out with the highest content could be due to overcooking (overnight) and the high dry matter content of these kernels. In agreement with Vodouhe *et al.* [34] works, on leafy vegetables, cooking process causes a sharp increase in the lipid content. This increase in lipid content may be due to the water loss and of dry matter concentration. Total carbohydrates (as calculated by difference) were very low (5.6-13.1%), with low amounts of sugar (2.0-2.7%) irrespective of the production areas considered. Similar levels of carbohydrate were observed in the seeds kernels of *R. heudelotii* collected at Gagnoa region's in Cote d'Ivoire [35] or at Yaoundé in Cameroon [29]. It was not surprising since several previous studies have shown that the *R. heudelotti* seeds are naturally known for being very poor in carbohydrate [35, 5]. Our result thus gives us an indication that the energy source is largely lipids and in some extent protein (through deamination). For total dietary fiber, Lakota was the region with the lowest value (8.2%), but the value of 10.8% (Akpi-Bon) obtained to Bondoukou was higher than that in raw lentils seeds (6.3 g/100 g by fresh weight) [36]. From a nutritionally point of view, the range of fiber content recorded is advantageous as fiber in food is essential in decreasing cholesterol and blood sugar. It absorbs water and provides roughage for the bowels, assisting intestinal transit [37]. The calculated metabolizable energy values of kernels presented in Table 1 ranged from 577.4 kcal/100 g (Akpi-Vav) to 618.6 kcal/100 g (Akpi-Div). These values are close with previous investigations [35, 5] and are greater than those found by CIFOR [33]. The differences observed in these samples could be due to the difference in protein, lipid and fiber reported in proximate composition. Indeed, both lipid (47.8% - 54.8%) and protein level (23.4-28.3%) were the highest contributors to calories from the determination of energy produced. The carbohydrate contents did not considerably affect the determination of energy. The residual moisture content of kernels varied from 2.8% (Akpi-Div) to 4% (Akpi-Agb2) and was slightly lower than what was reported by the literature [29, 35, 7]. For Hong *et al.* [38], food products with moisture content of 3.1 to 8.7% have water activity less than 0.7. This explain why their seeds kernels can be stored for a long time with minimum chemical degradation and microbial contamination [39]. A significant differences remain ($p \leq 0.05$) especially,

between the production process of Divo region's (2.8%) and other regions (3.9% in mean; $p \geq 0.05$). A result could be attributed to its preparation steps, such as a solar drying time. He differs from one diagram to another and to the environmental conditions (Figure 2). The ash content of samples ranged from 5.3% (Akpi-Div) to 6.8% (Akpi-Vav). These levels vary from one process to another and are slightly lower with ash values of 7.5% reported by Kouamé *et al.* [35], but also are closely low with ash values of 3.7%, 3.2% and 3.6% reported for pigeon pea, lima bean and lablab bean, respectively [40]. Ash content is an indicator for mineral elements [31]. Thus, from the result it could be seen that Akpi-Vav sample with 6.8% was the best in terms of mineral content. The minor ash variation in kernels could be due to the geographical origin of the samples, the climatic conditions and the edaphic characteristics of soils [41]. It has been recommended by Pomeranz and Clifto [42] that ash contents of seeds and tubers should be in the range 1.5-3.5% in order to be suitable for animal feeds and human consumption. In this study, the ash content fall within this range hence it can be recommended for animal feeds and human consumption. For all the samples, pH was slightly acid (5.2-6.4), while total acidity was low (2.6 to 4.8 meq / 100 g). This may confer longer keeping quality of them [43]. Total acidity in the samples are consistent with the observations of Saki *et al.* [5]. These authors reported values between 2.8 - 3.1 meq/g. From the statistical analysis, both total titrable acidity and pH showed that all the samples were significantly different ($p \leq 0.05$) and the differences observed may be related to the storage process, which is a function of the drying step [44]. According to Treche *et al.* [44], the drying time has a significant influence on the acidity of the flour. A long drying time would reduce the titratable acidity of the flours. The determinations of titratable acidity, pH and oxalic acid is of interest because of their alleged adverse effect on mineral bioavailability.

Table 1. Proximate composition of kernels flours from *R. heudelotii* (Bail.)

	Akpi-Agb1	Akpi-Agb2	Akpi-Div	Akpi-Lak	Akpi-Bon	Akpi-Vav
Moisture (%)	3.9±0.1^{a}	4.0±0.1^{a}	2.6±0.0^{b}	3.7±0.1^{a}	3.9±0.1^{a}	3.9±0.0^{a}
TS (%)	2.6±0.1^{a}	2.8±0.1^{a}	2.2±0.0^{a}	2.3±0.0^{a}	2.1±0.1^{a}	2.0±0.2^{a}
SC (%)	0.2±0.0^{a}	0.1±0.0^{a}	0.1±0.0^{a}	0.1±0.0^{a}	0.1±0.0^{a}	0.1±0.0^{a}
Ash (%)	6.2±0.0^{a}	6.7±0.0^{b}	5.3±0.0^{c}	6.2±0.0^{a}	6.1±0.0^{a}	6.8±0.0^{b}
Protein (%)	23.4±0.0^{a}	28.3±0.1^{b}	25.6±0.0^{c}	24.3±0.0^{d}	24.5±0.0^{d}	23.7±0.0^{a}
Lipid (%)	51.4±0.0^{a}	49.4±0.1^{b}	54.8±0.0^{c}	51.0±0.1^{a}	49.1±0.1^{b}	47.8±0.0^{d}
TCHO (%)	9.7±0.1^{a}	5.6±0.2^{b}	5.8±0.1^{b}	10.3±0.1^{a}	9.5±0.1^{a}	13.1±0.0^{c}
Crude fibre (%)	9.3±0.0^{a}	9.9±0.0^{b}	8.5±0.0$^{c.d}$	8.2±0.0^{c}	10.8±0.1^{e}	8.6±0.0^{d}
pH	6.2±0.0^{a}	5.5±0.0^{b}	5.9±0.0^{c}	5.2±0.0^{d}	5.6±0.0^{b}	6.4±0.0^{a}
TTA (meq/100 g)	3.6±0.0^{a}	4.8±0.0^{b}	3.1±0.0^{c}	3.2±0.0^{d}	4.1±0.0^{b}	2.6±0.0^{a}
Energy (kcal/100g)	594.8±0.1^{a}	580.4±0.6^{b}	618.6±0.1^{c}	597.5±0.4^{a}	578.1±0.2^{b}	577.4±0.2^{b}

Data are represented as Means ± SD (n = 3). Means in the lines with no common superscript differ significantly ($p<0.05$) according to Duncan's test.; total sugars: TS. Total carbohydrates: TCHO. TTA: Total Titratable Acidity. Akpi-Lak = Akpi of Lakota; Akpi-Bon = Akpi of Bondoukou; Akpi-Agb1 = Akpi d'Agboville1; Akpi-Agb2 = Akpi d'Agoville2; Akpi-Vav = Akpi of Vavoua; Akpi-Div = Akpi of Divo

Table 2. Mineral composition of kernels flours from *R. heudelotii* (Bail.)

	Akpi-Agb1	Akpi-Agb2	Akpi-Div	Akpi-Lak	Akpi-Bon	Akpi-Vav
Mg (%)	0.9 ±0.1^{a,b}	1.0±0.1^{c}	0.9±0.1^{a,b}	0.9±0.0^{a}	1.0±0.0^{b,c}	1.2±0.1^{d}
P (%)	2.0±0.2^{a}	2.2±0.0^{b}	1.8±0.1^{c}	1.9±0.0^{a,c}	2.2±0.1^{b}	2.3±0.0^{d}
Cl (%)	0.0±0.0^{a,b,c}	0.0±0.0^{c}	0.0±0.01^{b}	0.0±0.0^{a,b}	0.1±0.0^{a,b}	0.0±0.0^{a,c}
Ca (%)	1.8±0.2^{a}	1.7±0.2^{a,b}	1.5±0.1^{c}	1.4±0.2^{c}	1.6±0.1^{b,c}	1.7±0.1^{a,b}
K (%)	0.6±0.1^{a}	0.8±0.2^{b}	0.6±0.1^{a}	0.7±0.0^{a,b}	0.9±0.2^{c}	0.7±0.1^{b}
Fe (%)	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
Zn (%)	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}

Data are represented as Means ± SD (n = 3). Means in the lines with no common superscript differ significantly ($p<0.05$) according to Duncan's test. Akpi-Lak = Akpi of Lakota; Akpi-Bon = Akpi of Bondoukou; Akpi-Agb1 = Akpi d'Agboville1; Akpi-Agb2 = Akpi d'Agoville2; Akpi-Vav = Akpi of Vavoua; Akpi-Div = Akpi of Divo; K: Potassium; P: Phosphorus; Ca: Calcium; Mg: Magnesium; Fe: Iron; Zn: Zinc; Cl: Chlorine

Secondly, we evaluated some mineral of *R. heudilotii* seeds kernels, in order to assess the nutritional quality of the ash fraction. Minerals are important components of diet because of their physiological and metabolic function in the body. Table 2 shows the concentration of mineral elements in *R. heudilotii* seeds kernels for the various locations. Overall, the mineral and trace element contents differed significantly between seeds kernels samples excepting iron and zinc content. These finding could be explained because of unit operations on the extraction kernels of the *R. heudelotti,* but also by differences in the mineral contents between the soils of sites [45]. In this study, *R. heudilotii* seeds kernels were moderately a sources of dietary minerals. Among the microelements, the predominant mineral in the kernels was phosphorus (1.8 - 2.3%). Notable contributions to the intakes of calcium (1.4 - 1.8%), magnesium (0.85 - 1.15%), potassium (0.6 - 0.9) and chlorine (0.02 - 0.05%) were also found, while both iron and zinc contents (0.01%) were similar and that, whatever producing areas. Mineral contents of *R. heudilotii* seeds kernels reported in the literature are often different. The highest phosphorus content is found in Akpi-Vav (2.3%) due to relatively less intense firing than the other kernels (Figure 1). This value is high compared to those of 1.7% reported by Saki *et al.* [5]. The calcium content assessed was higher compared to that reported in literature [33, 5]. Variations in calcium content could be due to calcium intake or no, from cooking water through the crack in the hull. According to Lestradet and Machinot [46], boiling heavily increases the calcium intake during the cooking. The magnitude of the difference depends on the temperature and the duration of treatment. The magnesium content found revealed significant variability, with respect to traditional extraction process of seeds. It was well above the value of 0.2% obtained by Saki *et al.* [5], while the potassium content found in the kernels collected was within the range reported in the literature [33, 5]. The difference recorded could be also related to the cooking time. Chlorine was detected in our samples. The lowest levels of chlorine were found in the kernels relatively to their production process. This variation may be as a result of difference different soil types or whether fertilizer is ingested or not. Other studies found lower amounts of chlorine in the seeds kernels of *R. heudilotii* [5]. The trace elements levels (iron and zinc) detected in kernels were very low, compared than values (8 and 6 mg/day, respectively) recommended for human dietary allowance [47]. The traditional kernels extraction process did not affect the content of these components (Table 2).

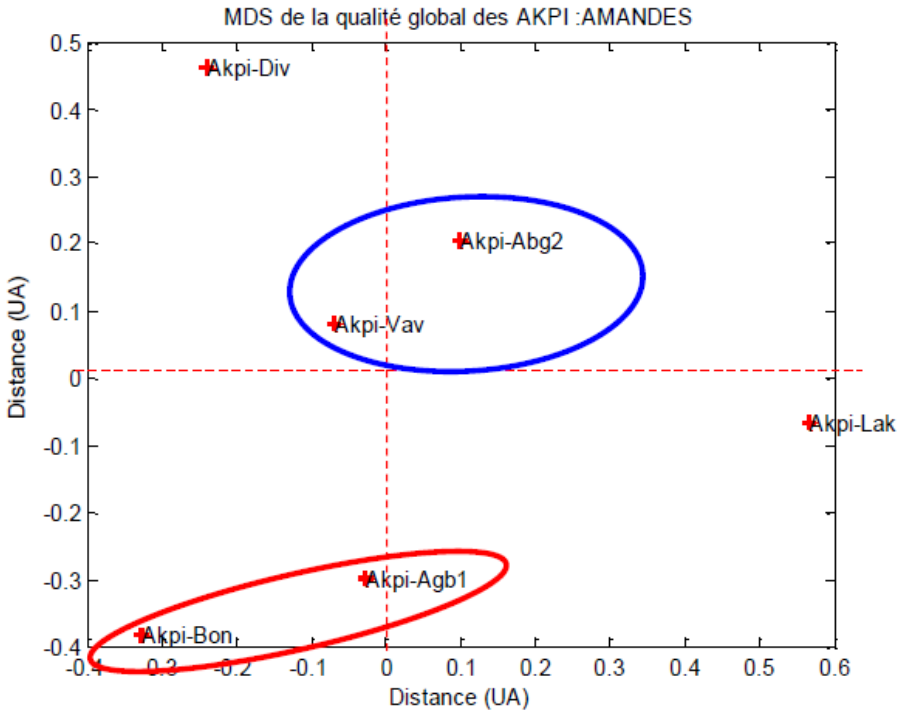
Finally, we evaluated some of the nutritive and antioxidant properties of *R. heudilotii* seeds kernels (Table 3). The result of the analysis revealed an appreciable amount of phytochemicals such as total polyphenols, phytates, flavonoids, tannins and oxalates. Noticeable differences were observed between samples for all the components measured ($P < 0.05$) with the exception of oxalate. Indeed, there were high variability with respect to traditional extraction process. The polyphenols are the main dietary antioxidants among selected *R. heudilotii* seeds kernels. In our samples, the total polyphenols content ranges from 54.56 mg/100g (Akpi-Agb1) to 156.8 mg/100g (Akpi-Bon) and was very high when compared to *R. heudelotii* seeds collected in local food spice market in Port Harcourt, Rivers state, Nigeria (0.002 mg/100g) [48]. On the other hand, these amounts are lower than those found in vegetable foods (2545 – 3552 mg/100g) as *Moringa oleifera* leaves [49]. Following the example of previous studies that tested fruits from tropical regions for their polyphenol contents [50], all seeds kernels samples of *R. heudilotii* evaluated, in this study can be categorized as having a low concentration of phenolic compounds (< 500 mg/100 g), consequently with a low source of phenolic compounds. As postulated by Mehinagic *et al.* [51], the total polyphenol content depends not only on the extrinsic factors (geographical and climatic factors), but also the genetic factors, the degree of maturation of the plant, storage time and the technical route have strong influence on the content of polyphenols. Compared to the amounts found by Odinga *et al.* [48], the concentration of flavonoids was equally high. Seeds kernels of *R. heudilotii* collected in Agboville1 (6.7 mg/100 g) had the highest levels of flavenoids followed by those collected in Agboville2 (4.4 mg/100 g), Divo (4.1 mg/100 g); Bondoukou (3.0 mg/100 g) and Vavoua (1.3 mg/100 g) in that order. These differences can be explained as not only due to differences in producing areas of the samples but also due to the seeds kernels extraction systems applied. This fact is also was also found by Bolanho and Beléia [52]. Flavonoids exhibits a range of biological activities, one of which is their ability to scavenge for biological radicals and superoxide anions radicals and thus health promoting in action [53]. On the other hand, it was also observed that the selected *R. heudilotii* seeds kernels used in this study contained anti- nutrients, with values ranging from 382.6 mg/100 g (Akpi-Bon) to 593.3 mg/100 g (Akpi-Vav) for oxalates, 6.8 (Akpi-Agb2) to 34.9 mg/100 g (Akpi-Vav) for tannins and 2.0 (Akpi-Agb1) to 25.8 (Akpi-Bon) for phytates. Their presence in the samples is of significant importance since they have some deleterious effects on both human health and other animals. For instance, oxalate is a chelating agent, which binds calcium very effectively [31]. Although the mineral content of *R. heudelotii* seeds was were seen in appreciable concentration, the oxalate content was high, but did not differ significantly from one extraction process to another. The amounts found in the kernels are similar to other plants rich in this anti-nutritional factor (430 - 1050 mg/100 g) [54], but lower than those found in legumes [55]. They are greater than those found in Nigeria (0.001 mg/100 g) [48]. These differences may be explained by several factors such as processes of harvesting of seeds or kernels extraction methods of *R. heudelotii*. In all cases, it is now know that plants with high oxalate content may produce acute metabolic calcium deficiency (hypocalcemia) when we use plant product as a main food source [31]. The phytate amount of kernels in this study was very low compared to 87-126 mg/100 g found by Reddy [56] in soybeans. The lowest concentrations of

phytate could be advantageous to the health status of consumers. Indeed, phytates are anti-nutrients which chelate divalent cations such as Zn^{2+}, Fe^{2+}, Fe^{3+}, Ca^{2+}, Mg^{2+}, Mn^{2+} and Cu^{2+}, thereby reducing their bioavailability. The few deviations in the data could be related to the precooking step in the processes that would resemble dipping steps. According to the FAO [57], the practice of soaking considerably reduces the phytate content of legumes. Tannins had been reported to affect protein digestibility, adversely influencing the bioavailability of nonhem iron leading to poor iron and calcium absorption, also carbohydrate is affected leading to reduced energy value of a diet containing tannins [58]. However, it's anti-nutritional/toxicity effects depend upon their chemical structure and dosage [59]. In the case of our samples, the tannin level was found to be relatively high in comparison with tannic acid found in some literatures [48]. The differences between them probably indicates a beneficial effect on total tannin content due to cooking process. This fact is also corroborated by those mentioned by Mezajoug [60]. This author showed that, long cooking times (90-120 min) reduced the tannin content significantly. The sampling influenced the data in many of the studied parameters, however, no conclusions can be made because other factors such as site of production or cultivation cycle also had an effect and therefore limits the interpretation. Thus, other multivariate statistical techniques, such as MDS was applied to obtain better and more consistent conclusions by means of a multiple comparison among the chemical compositions of each oilcakes sample.

Table 3. Bioactive compounds content of kernels from *R. heudelotii* (Bail.)

	Polyphenols (mg/100g)	Phytates (mg/100g)	Flavonoids (mg/100g)	Tannins (mg/100g)	Oxalate (mg/100g)
Akpi-Agb1	156.8±0.6^{a}	2.1±0.0^{a}	6.7±0.4^{a}	27.4±0.3^{a,b}	492.7±0.2^{a}
Akpi-Agb2	128.1±0.7^{b}	10.3±0.0^{b}	4.4±0.1^{b}	6.8±0.2^{c}	451.0±0.2^{a}
Akpi-Div	73.7±0.2^{c}	7.2±0.1^{c}	4.1±0.1^{b}	16.5±0.9^{a,c}	412.3±0.1^{a}
Akpi-Lak	61.3±0.1^{d}	19.0±0.1^{d}	1.3±0.1^{c}	19.8±0.4^{a,c}	413.0±0.2^{a}
Akpi-Bon	54.6±0.1^{d}	25.8±0.0^{e}	3.0±0.0^{b,c}	15.1±0.4^{a,c}	382.6±0.1^{a}
Akpi-Vav	117.0±0.5^{b}	22.7±0.1^{f}	1.3±0.1^{c}	34.9±0.2^{b}	593.3±0.2^{a}

Data are represented as Means ± SD (n = 3). The values within a column with different superscript letters are signifcantly (P < 0.05) different according to Duncan's test.; Akpi-Lak = Akpi of Lakota; Akpi-Bon = Akpi of Bondoukou; Akpi-Agb1 = Akpi of Agboville1; Akpi-Agb2 = Akpi of Agoville2; Akpi-Vav = Akpi of Vavoua; Akpi-Div = Akpi of Divo



Akpi-Lak=Akpi of Lakota; Akpi-Bon=Akpi of Bondoukou; Akpi-Agb1=Akpi d'Agboville1; Akpi-Agb2=Akpi d'Agoville2; Akpi-Vav=Akpi of Vavoua; Akpi-Div=Akpi of Divo;Akpi-Lak = Akpi of Lakota; Akpi-Bon = Akpi of Bondoukou; Akpi-Agb1 = Akpi d'Agboville1; Akpi-Agb2 = Akpi d'Agoville2; Akpi-Vav = Akpi of Vavoua; Akpi-Div = Akpi of Divo

Figure 3. Multidimensional scaling analysis kernels of *R. heudelotii* (Baill) Pierre Pax (Euphorbiaceae) resulting from different production processes of kernels according to their main biochemical characteristics

3.1. Multidimensional Scaling (MDS) Analysis

By performing principal coordinate analysis, also known as multidimensional scaling, the MDS plot presented in Figure 3 was obtained. Multidimensional scaling (MDS) projects a distance matrix into a set of coordinates such that the Euclidean distances of these coordinates approximate the original distances [61]. The distances between the objects depicts their (dis)similarities: similar objects are represented by points that are closer, and dissimilar objects by points that are further apart. The dimensions (Distance UA in figure 3) are factors with real meaning that make it possible to explain the differences among groups. The MDS plot (Figure 3) shows that the *R. heudelotii* seeds kernels samples were clearly classified into two groups and an individual sample according to seeds kernels extraction systems and physico-chemical characteristics: Agboville1-Bondoukou diagram (Akpi-Bon; Akpi-Agb1, code sample); Agboville2-Vavoua diagram (Akpi-Agb2; Akpi-Vav; code sample) and an individual samples (Divo diagram and Lakota diagram).

Considering the first group composed of Akpi-Bon and Akpi-Agb1 (Figure 3), it can be observed that the kernels production processes of Bondoukou and Agboville1 show strong similarities separate quadrants. The similarity attributed to potassium (0.71198), iron (0.88641) and zinc (0.77142) is observed in similar trends (Table 2). This similarity is confirmed by the variance analysis for these minerals which shows that there is no significant difference between the contents ($p > 0.05$) (Table 2). This similarity is also expressed by the F1 distances expressed to the level in proteins (0.84032), lipids (0.83761), fiber (0.94313), pH (0.72519), titrable acidity (0.97630) and energy value (0.91884). Characteristic distances of similarity agree with data of Tables 1 and 2. Although these two kernels categories are located in a similar geometric space, they show significant distances for polyphenols (0.97738), phytates (0.95211) and flavonoids (0.74872). These observations suggests that the extraction process of kernels from Agboville1 and Bondoukou are similar reading from the diagram. As for the second group composed of kernels from Agboville2 (Akpi-Agb2) and Vavoua (Akpi-Vav), only one mineral substance, phosphorus (0.79019) was characteristic of this resemblance. They are higher than the average of 1.7% obtained by Saki *et al.* [5]. Their rapprochement on the graph is supported by similar trends such as proteins (0.97057), lipids (0.92081), total carbohydrates (0.98848), fibers (0.92621), pH (0.97270) and titrable acidity (0.93506), a biochemical parameters. Similarity is also characterized by the distances observed in the polyphenols (0.85947), phytates (0.95594), flavonoids (0.90933) and tannins (0.72511). Because of their negative F1 factor, these parameters show significant distances despite the close spatial position of the kernels on the graph. This is mainly demonstrated by the average phytate contents (10.3 ± 0.7; 22.7 ± 1.1 mg/100 g), flavonoid (4.40 ± 0.87; 1.34 ± 1.11 mg/100 g) and tannin 6.81 ± 1.53; 34.94 ± 17.03 mg/100 g). These results explain why the extraction process of kernels of Agboville2 and Vavoua are similar. The kernels from the Divo diagram are separate from the other kernels. The dimensions of the MDS outlet characteristic of this remoteness are attributed to the minerals phosphorus, chlorine and potassium as well as dry matter and ash. The distances expressed by the F1 factors of these parameters agree with the mean contents of these parameters for these kernels. These kernels have the lowest phosphorus content (1.8 ± 0.1%), potassium (0.6 ± 0.1%), ash (5.3 ± 0.0%), and higher chloride content (0.1 ± 0.0%), dry matter (97.2 ± 0.2%). The anti-nutritional substances quantified are also characteristic of the kernels dissimilarity resulting from the Divo diagram compared to the other kernels. This is confirmed by the polyphenols (73.74 ± 23.20 mg/100 g) and phytates (7.18 ± 0.95 mg/100 g) contents, which represent some of the lowest levels of these substances. The last group constituted by the Lakota diagram distinguishes from other groups by its own biochemical characteristics. The MDS output dimensions, characteristic of this dissimilarity are attributed to most quantified minerals as magnesium, phosphorus, chlorine, potassium, iron and zinc. F1 distances of these minerals are reinforced by the data of Table 2, where a very low average values were recorded. The dissimilarity with other kernels was also characterized by the F1 dimensions observed for dry matter, total sugars and reducing sugars, total carbohydrates, pH, energy, phytate, flavonoids, tannins and oxalates. These distances are shown mainly by the results obtained for the pH. This pH (5.2), which is the lowest, makes it possible to discern this difference between the kernels. The results of variance analysis for this parameter confirm the existence of significant difference between the kernels of Lakota and those of the other regions ($p \leq 0.05$).

4. Conclusions

The results in the present study reaffirm that seed kernels of *R. heudelotii* has higher nutrient composition and calorie value compared to some legumes most especially in terms of crude oil and protein. From the result, the kernels extraction techniques employed may influence the physicochemical composition and antioxidant properties of seeds kernels. In particular, the contents of crude protein, crude fat, oxalate, phytate and ash were highly variable. MDS identify the relevant chemical compounds responsible for the main differences among samples and extrinsic factors such as geographical location (locality of production) and kernels extraction systems. These results provide useful indications of the effect of traditional extraction process on the physico-chemical and antioxidant properties of kernels flours from *R. heudelotii.*

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