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# Effect of Post-Harvest Traditional Technologies on the Nutrient Content and Antioxidant Compounds of Defatted Flours from *Ricinodendron heudelottii* (Baill. Pierre ex Pax) Seed Kernels

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**Abstract:** *Akpi* (*Ricinodendron heudelottii*) is a semi-deciduous plant species indigenous to the coastal nations of West Africa. Its kernels are rich in nutrients and bioactive compounds. In this article, we performed a nutritional characterization and a phytochemical composition of defatted kernel flours from *Ricinodendron heudelottii* that were grown in six different regions of Cote d'Ivoire. Classical analytic methods were used to determine the chemical composition. There were significant differences between the samples for all the parameters studied; in addition, both the locality of production and the *Ricinodendron heudelottii* seed kernels extraction systems showed a noteworthy influence on the mineral and element composition of the defatted flours. The samples were also rich in protein (47.0–61.3%) and energy (330.4–339.2 kcal/100 g). Magnesium (12.0–40.0%) was found to be the predominant mineral. Polyphenols (216.6–403.9 mg/100 g) and oxalates (714.7–972.9 mg/100 g) were the main phytochemical compounds identified. The consumption of defatted kernel flours from *Ricinodendron heudelottii* may contribute relatively high intake levels of protein, carbohydrate, and mineral macro- and micronutrient (K, Mg, Ca, Mn, and Fe). By performing principal coordinate analysis, the multidimensional scaling plot classified the defatted flours according to the kernel extraction into four main extraction systems: Bondoukou-Lakota-Vavoua diagram, Agboville1 diagram, Divo diagram, and Agboville2 diagram.

**Keywords:** *Akpi*; antioxidant compounds; food analysis; food composition; multidimensional scaling; mineral and trace elements; *Ricinodendron heudelottii*; traditional extraction method

## 1. Introduction

Among the species of non-timber forest products (NTFPs), several wild fruits and vegetables are consumed in Africa and particularly in Cote d'Ivoire (West Africa) [1]. These wild fruits and vegetables contribute significantly to covering rural nutritional needs and represent a source of income for the household [2]. One of these wild fruits and vegetables is the *Ricinodendron heudelottii* (Baill. Pierre ex Pax), a member of the Euphorbiaceae family. The plant is known to have two subspecies, namely, *heudelottii* and *africanum* (Müller of Aargau, Müll. Arg.) [3]. Subspecies *heudelottii* is known to occur

in Senegal and Benin, while the subspecies *africanum* is located in the southern part of Nigeria and South Africa. The subspecies *Ricinodendron heudelottii* (*R. heudelottii*) is the only one encountered in Cote d'Ivoire. *R. heudelottii* species 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 tropics. 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 Cote 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]. As for bioactive compounds from the seeds of *R. heudelottii*, their defatted flours contain 940.0 mg/100 g of polyphenolic compounds, 17.57 mg/100 g phytates, and 2690.9 mg/100 g of oxalates and may be responsible of the numerous pharmacological properties [6]. From a nutritional point of view, the amino acids of protein from *R. heudelottii* defatted kernel flour are of the same level according to Tchiégang et al. [7]. In the human diets, proteins are obtained from animal and plant sources, and *R. heudelottii* defatted flour can be used as an alternative source of protein from plant origin as much as cereal and legumes [8]. Several kernel extraction technologies exist independently of production areas. The kernels from *R. heudelottii* fruits, widely consumed, arise out of the ability of 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 are extracted after washing and boiling steps. An additional boiling of the seeds is then necessary to soften them and enable cracks to appear in the shells. After this, the kernel is removed from the seed using a knife or any other sharp thing. The kernels are dried in the sun or in an oven and can be stored for several years. They may be sold throughout the year in urban markets [9]. Studies of post-harvest and processing technologies for fruits, nuts, and kernels showed that the kernel extraction system is one of the chemical and nutritional property factors of food variation [10]. Despite the increasing number of scientific reports on the *R. heudelottii* [11–13], studies of the overall nutritional quality of defatted seeds from Cote d'Ivoire are few. With the aim of valorising defatted flours, knowledge of its nutrients and some anti-nutrient composition with respect to the traditional extraction process need to be generated. The objectives of this study, therefore, was to investigate the nutrients, minerals, phytochemical, and bioavailability of defatted flours from *R. heudelottii* seed kernels grown in six producing areas of Cote d'Ivoire. In this study, the effect of the traditional kernel extraction process on the physico-chemical and antioxidant compounds of defatted flours from *R. heudelottii* was assessed.

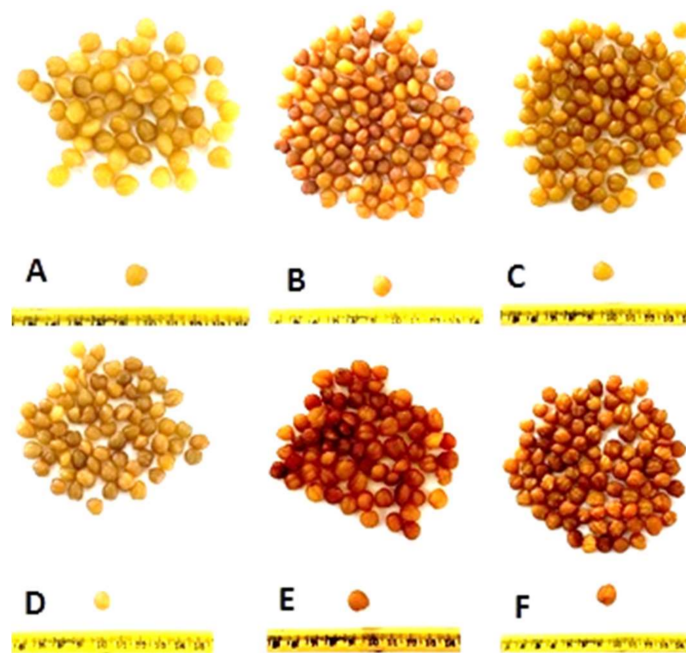
## 2. Materials and Methods

### 2.1. Materials Chemicals and Reagents

All chemicals and reagents from Sigma Chemical Co. (St. Louis, MO, USA) were of analytical grade. Ultra-pure water of 18.2 M $\Omega$  resistivity was prepared by ELGA water purification system (Flex III, Buckinghamshire, UK) and used in all experiments.

### 2.2. Sampling and Sample Preparation

The plant material was mainly the kernels of *R. heudelottii* (Figure 1). Thus, fresh *R. heudelottii* kernels were collected from six different producing areas of Cote d'Ivoire (West Africa): Bondoukou, Agboville1, Agboville2, Divo, Lakota, and Vavoua. The unit operations involved in each method 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, Cote 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 matter was ground into fine flour using a laboratory blender (Bimby mod. 2200, Vorwerk, Wuppertal, Germany). Fine flour obtained was defatted for 12 h with hexane using Soxhlet apparatus [14]. All analyses were carried out in three repetitions on equal amounts of samples.



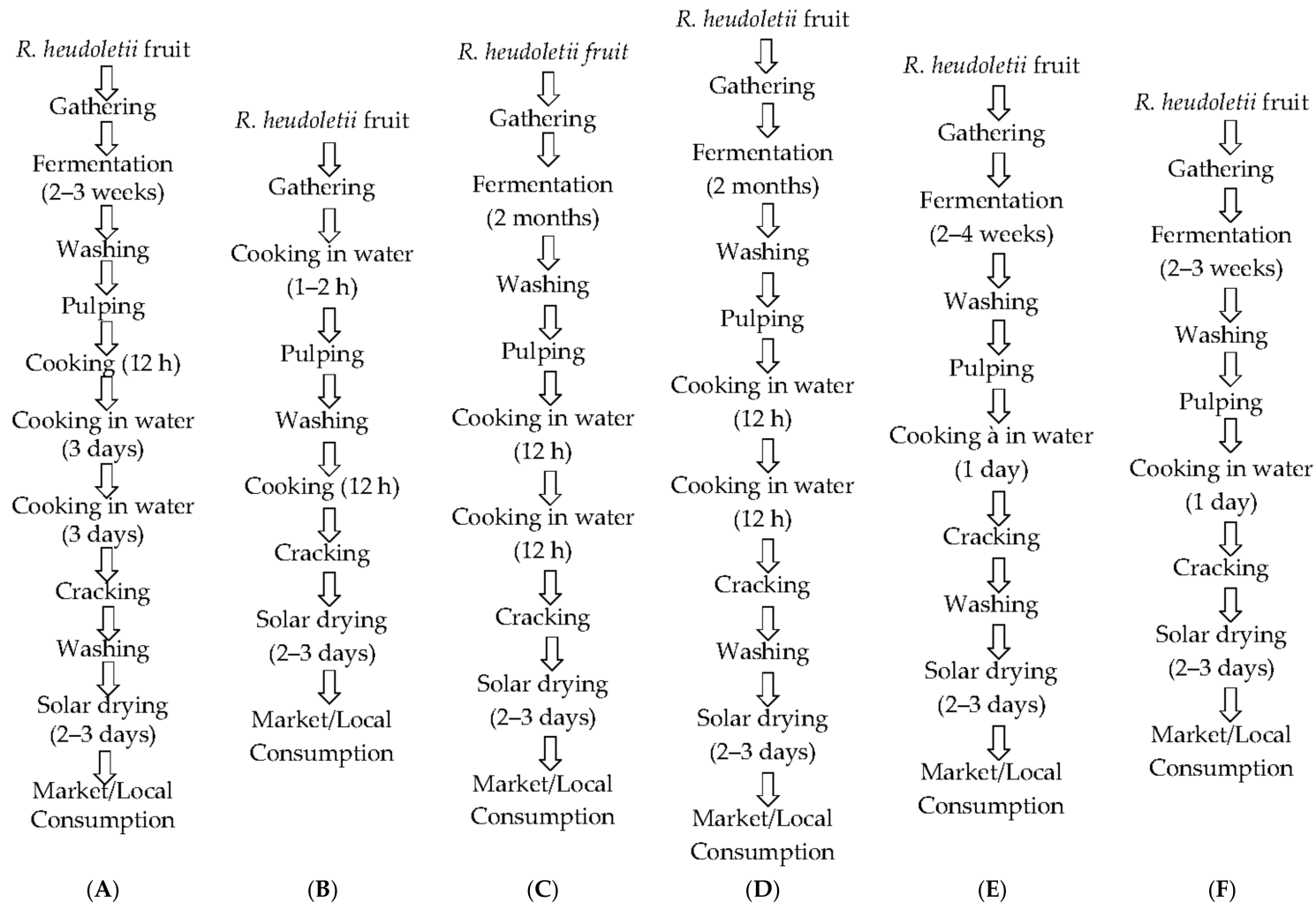
**Figure 1.** *Ricinodendron heudelottii* seed kernel samples from “Bondoukou” (A), “Agboville2” (B), “Divo” (C), “Lakota” (D), “Vavoua” (E) and “Agboville1” (F).

### 2.3. Proximate Analysis of Defatted Flours from *R. heudelottii* Seeds Kernels

The methods used for sample treatment and analysis (Moisture, ash, protein) were carried out following standard procedures recommended by Association of Official Analytical Chemists (AOAC) [14]. Moisture was determined by gravimetric method and was heated in an oven at  $105 \pm 1$  °C until it became a constant mass. Total nitrogen was determined by the Kjeldahl method and converted into protein using factor 6.25. Ash was determined by gravimetry of incinerated sample, in muffle, at 550 °C. The total carbohydrates (TCHO) were calculated by difference as suggested by Food and Agriculture Organization/World Health Organization (FAO/WHO) procedure [15]. The total dietary fiber content was determined following Prosky’s protocol [16]. Total sugars (TS) were determined using the phenol-sulphuric acid method [17], while the reducing sugars (RS) were quantified by the oxido-reduction method using 3,5-dinitrosalicylic acid (DNS) as oxidizing agent [18]. The energy content was estimated by multiplying each gram of carbohydrate, protein, and lipid by 4 kcal, 4 kcal, and 9 kcal, respectively [19].

### 2.4. Mineral Composition of Defatted Flours from *R. heudelottii* Seeds Kernels

The sample was accurately weighted and dry-ashed (550 °C, one night; method 40–70.01) [20] in a muffle furnace (Cavallo Srl, Buccinasco, Italy). Grey ashes were treated with high purity hydrogen peroxide (H<sub>2</sub>O<sub>2</sub> 30% Suprapur, St. Louis, MO, USA) to obtain white ashes, which 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 Spectroscopy (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) [21]. All analyses were performed in triplicate with two measurements per analysis. All minerals were reported as % dry weight.



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

### 2.5. pH Determination of Defatted Flours from *R. heudelotti* Seeds Kernels

The pH of samples was measured by use of a pH meter, model 744 (Metrohm AG, Herisau, Switzerland) following to the procedure of the Swiss Food Manual (Schweizerisches Lebensmittel-Buch, 2001). The pH meter was calibrated with standard buffers 4 and 7, before the pH was measured. The results are expressed in pH units and are read directly on the pH meter dial.

### 2.6. Total Titrable Acidity (TTA) of Defatted Flours from *R. heudelotti* Seeds Kernels

The acidity of kernels was determined by titration following the method described by AOAC [14] 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.

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

### 2.7. Phytochemical Analysis of Defatted Flours from *R. heudelotti* Seeds Kernels

#### 2.7.1. Total Flavonoids Determination

The flavonoid assay was performed according to the method described by Meda et al. [22]. Briefly, a distilled water (5.6 mL) was added to 1 mL of defatted kernels flours of *R. heudelotti* extracts. Then, 3 mL 95% ethanol (*v/v*) was added, followed by 10% aluminium chloride solution (0.2 mL) and 0.2 mL of 1 mol/L potassium acetate. In a like manner, the amount of 10% (m/V) aluminum chloride was substituted by the same amount of distilled water in the blank. For assay of flavonoids, the absorbance was measured at 415 nm using a UV-VIS spectrophotometer (Cary 50 Bio, Varian Australia Pty. Ltd., Victoria, Australia) after incubation at room temperature for 30 min. A standard range established from a stock solution of quercetin (0.1 mg/mL) under the same conditions as the test determines the amount of flavonoids in the sample. Thus, flavonoids in defatted flours from *R. heudelotti* 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. [23]. 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 5 mL of the supernatant obtained are added 3 mL of 1% iron chloride prepared in hydrochloric acid HCl (1 M), which is then heated in a water bath for 45 min. After cooling the mixture, 5 mL of hydrochloric acid (HCl) is added and the mixture is then left to stand for 2 h. 5 mL of 1.5 M sodium hydroxide is then added to the mixture obtained above; the whole is carried in 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 and 4.5 mL of ortho-phenanthroline reagent. The optical density is read at 470 nm on the spectrophotometer against a blank. A reference range was established from a stock solution of Mohr salt (iron 10 µg iron/mL) under the same testing conditions as those for determining the amount of ferric phytate-sample. The amount of phytate content was expressed as mg/100 g dry weight.

#### 2.7.3. Tannins

The estimation of the extracted tannins was carried out according to the method described by Bainbridge et al. [24]. To determine tannins in defatted flours from *Ricinodendron heudelotti*, two grams of sample with 30 mL of 80% acetone were mixed in 50 mL Erlen-meyer flasks. The mixture was shaken for 15 min and filtered under pressure through a sintered glass using a Buchner. Acetone was then removed by evaporation using a rotary evaporator (Buchi Rotavapor R-124, Buchi Labortechnik, Flawil, Switzerland). The extracts obtained were diluted 20 times with distilled water. 1 mL of diluted extract was introduced into test tubes covered beforehand with aluminum foil to exclude the light,

follow-up of 3 mL of a coldly prepared vanillin 4% solution in the ethanol (*p/v*). Each test tube is agitated and 1 mL of concentrated HCl was added in each tube which thereafter was left at rest at room temperature for 15 min. Thereafter, the absorbency was measured photometrically (Spectrophotometer T80 + UV/Vis Spectrometer, PG Instruments Ltd, Leicestershire, United Kingdom) at the wavelength of maximum absorption of 500 nm against a blank solution (without extract). Catechin was used as a reference standard. 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

The method described by Day and Underwood [25] was used to determine the oxalate content of defatted kernels flours of *R. heudelotti* extracts. This method consists of extracting the total oxalates (insoluble and soluble) followed by titrimetric analysis. Briefly, one gram of dried powdered and 75 mL of 1.5 N H<sub>2</sub>SO<sub>4</sub> were added in 100 mL conical flask. The mixture was carefully shaken on a mechanical shaker for 1 h. Then, the solution was filtered using Whatman No.1 filter paper. A 25 mL of filtrate was titrated hot (80–90 °C) with freshly prepared 0.1 N KMnO<sub>4</sub> solution until the colour of the solution become pink persisted for 30 seconds. The results were expressed as mg/100 g dry weight.

#### 2.7.5. Total Phenolic Assay

The phenolic extracts of defatted kernel flours of *R. heudelotti* were obtained following the procedure described by N'dri et al. [26] and quantified using the Folin-Ciocalteu assay [27]. Briefly, 10 mL of crude extract and 10-fold diluted Folin-Ciocalteu reagent were reacted with sodium carbonate at a concentration of 6% (*w/v*) into 25 mL of volumetric flask containing deionized water. Following a mean incubation period of 15 min and away from light, the mixture's absorbance against the reagent blank (the blank being prepared under the same conditions as before but without extract) was measured at 725 nm using a spectrophotometer (model T80 x UVNIS Spectrometer PG Instruments. Ltd, Leicestershire, United Kingdom). Total phenolic content was presented as mg gallic acid equivalent (GAE)/100 g sample.

#### 2.8. Statistical Analyses

The collected data were subjected to a statistical analysis carried out with the Statistica 9.0 software (StatSoft, Krakow, Poland). They were presented as standard deviation means (SD) and analyzed by Excel 2013 from Microsoft Corporation. The differences between the samples were revealed by analysis of variance (ANOVA). Duncan's multiple range test ( $p < 0.05$ ) was used to determine the significances within treatments. The similarity between extraction systems of the seeds kernels were examined by multidimensional scaling (MDS). In order to do so, it took a proximity matrix between each pair of samples in the set of chemical constituents. Proximity indicate similarity estimates (similar objects) or rather the opposite (different objects) and are simple Euclidean distances applied to the standardized data. One-way ANOVA test with Duncan's multiple range post hoc was used to compare similarity groups with significant differences among them when  $p \leq 0.05$ . 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 defatted flours from *R. heudelotti* kernels were assessed.

Firstly, we performed a nutritional characterization of defatted flour 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 defatted kernel flour samples for all the parameters studied, a result that could be attributed to their steps of preparation (post-harvest traditional technologies) and to the environmental condition. Proximate composition confirms that the defatted kernel flours from *R. heudelotii* have potentially high protein content to satisfy the protein

demand of the populations and animal foods. They contain a considerable number of proteins, which vary from 47.0% (Akpi-Vav) to 61.3% (Akpi-Lak), which is higher than protein rich foods such as quinoa [28], bambara groundnut [29], and cowpeas [30] seeds which range between 13.5 and 26.8%. Considering the same sample, defatted kernel flours from *R. heudelottii* are twice as rich in protein compared with almonds (22.4–24.9%) found in the work of Saki et al. [5] and would cover the protein needs of populations, especially in developing countries. The high concentration of protein can be attributed to the water loss and dry matter concentration after cooking, drying, and defatting kernels of *R. heudelottii*. The protein contents of samples obtained from Bondoukou (Akpi-Bon: 56.6%), Agboville1 (Akpi-Agb1: 57.0%), and Lakota (Akpi-Lak: 61.3%) sites were higher compared to 50.3% obtained by Tchiegang et al. [31], but those obtained at Agboville2 (Akpi-Agb2: 49.4%), Divo (Akpi-Div: 48.8%), and Vavoua (Akpi-Vav: 47.0%) were approximately similar. The intrinsic differences between proteins (Table 1) may be explained by the compositional changes following the degradation of other constituents during the cooking processes [32]. Indeed, according to Fabbrin [33], heating before cooking improves the nutritional value and the availability of nutrients, which could be the case in this study. Total carbohydrates (as calculated by difference) was moderate (20.5–37.8%).

**Table 1.** Proximate composition, energy, and pH values of defatted flours from *R. heudelottii* seeds kernels.

Composition	Defatted Kernel Flour Samples					
	Akpi-Agb1	Akpi-Agb2	Akpi-Div	Akpi-Lak	Akpi-Bon	Akpi-Vav
Dry matter (%)	94.3 ± 0.0 <sup>a</sup>	92.7 ± 0.0 <sup>b</sup>	95.6 ± 0.0 <sup>c</sup>	94.0 ± 0.0 <sup>a,b</sup>	93.7 ± 0.0 <sup>d</sup>	95.0 ± 0.0 <sup>e</sup>
TS (%)	7.8 ± 0.8 <sup>a</sup>	9.8 ± 0.1 <sup>a</sup>	12.9 ± 0.1 <sup>a</sup>	17.1 ± 0.2 <sup>a</sup>	13.6 ± 0.1 <sup>a</sup>	7.3 ± 0.6 <sup>a</sup>
RS (%)	0.2 ± 0.0 <sup>a</sup>	0.3 ± 0.0 <sup>a</sup>	0.3 ± 0.0 <sup>a</sup>	0.2 ± 0.0 <sup>a</sup>	0.2 ± 0.0 <sup>a</sup>	0.2 ± 0.0 <sup>a</sup>
Ash (%)	12.5 ± 0.1 <sup>a,b,c</sup>	12.8 ± 0.1 <sup>b,c</sup>	13.1 ± 0.0 <sup>c</sup>	12.0 ± 0.1 <sup>a</sup>	12.2 ± 0.0 <sup>a,b</sup>	11.2 ± 0.1 <sup>d</sup>
Protein (%)	57.0 ± 0.0 <sup>a</sup>	49.4 ± 0.1 <sup>b</sup>	48.8 ± 0.1 <sup>b</sup>	61.3 ± 0.1 <sup>c</sup>	56.6 ± 0.1 <sup>a</sup>	47.0 ± 0.0 <sup>d</sup>
TCHO (%)	24.7 ± 0.1 <sup>a</sup>	31.1 ± 0.8 <sup>b</sup>	33.8 ± 0.1 <sup>c</sup>	20.5 ± 0.0 <sup>d</sup>	24.9 ± 0.1 <sup>a</sup>	37.8 ± 0.1 <sup>e</sup>
Crude fibre (%)	10.0 ± 0.1 <sup>a</sup>	13.7 ± 0.0 <sup>b</sup>	12.5 ± 0.0 <sup>c</sup>	11.1 ± 0.0 <sup>d</sup>	14.4 ± 0.0 <sup>e</sup>	9.5 ± 0.0 <sup>f</sup>
pH	6.2 ± 0.0 <sup>a</sup>	6.1 ± 0.0 <sup>b</sup>	6.7 ± 0.0 <sup>c</sup>	6.5 ± 0.0 <sup>d</sup>	7.1 ± 0.0 <sup>e</sup>	6.8 ± 0.0 <sup>f</sup>
TTA (meq/100 g)	4.3 ± 0.0 <sup>a</sup>	4.5 ± 0.0 <sup>b</sup>	2.3 ± 0.0 <sup>c</sup>	3.1 ± 0.0 <sup>d</sup>	1.9 ± 0.0 <sup>e</sup>	2.4 ± 0.0 <sup>c</sup>
Energy (kcal/100g)	326.9 ± 0.4 <sup>a</sup>	322.0 ± 0.2 <sup>b</sup>	330.4 ± 0.1 <sup>c</sup>	327.4 ± 0.3 <sup>a</sup>	325.9 ± 0.2 <sup>a</sup>	339.2 ± 0.2 <sup>d</sup>

Data are represented as Means ± SD ( $n = 3$ ). (<sup>a,b,c,d,e,f</sup>) Means in the lines with no common superscript differ significantly ( $p < 0.05$ ) according to Duncan's test.; Total sugars: TS. RS: reducing sugars. Total carbohydrates: TCHO. TTA: Total Titratable Acidity. Akpi-Lak = Akpi of Lakota; Akpi-Bon = Akpi of Bondoukou; Akpi-Agb1 = Akpi of Agboville1; Akpi-Agb2 = Akpi of Agboville2; Akpi-Vav = Akpi of Vavoua; Akpi-Div = Akpi of Divo.

The carbohydrate total assessed in our samples is almost double when compared to that observed in other seed kernels of *R. heudelottii* seed that come from seven different departments in Cote d'Ivoire [5], but appears lower than that reported for legumes such as bean, chickpea, etc. With low amounts of total sugar (7.3–17.1%), reducing sugars of samples are yet very low (0.2–0.3%), irrespective of the production areas. Similar levels of sugar were observed in defatted flours of *R. heudelottii* collected by Mezajoug and Tchiegang [6]. It was not surprising since several previous studies have shown that the *R. heudelottii* seeds are naturally known for being very poor in carbohydrate [5]. On the other hand, both carbohydrates and protein results thus give us an indication that the energy source is largely carbohydrate and to some extent protein (through deamination). As for the fibre contents, our results were significantly higher than those of seed kernels of *R. heudelottii* (2.3–2.5%) from seven different departments in Cote d'Ivoire [5]. These results were similar to studies of Mezajoug and Tchiegang [6], Mezajoug et al. [34], and Tchiegang et al. [31], who also found 11.0%, 10.9%, and 13.9%, respectively. Vavoua was the region with the lowest value (Akpi-Vav: 9.5%), but the value of 14.4% (Akpi-Bon) obtained from Bondoukou is slightly lower than the fiber content of the Pistachios from Bronte in Italy (15.5%) that are considered by D'Evoli et al. [35] as a key component of a healthy dietary pattern. Defatted flours from *R. heudelottii* seed kernels could therefore be considered as a valuable source of dietary fiber and, by inference, a good source of cellulose. From a nutritional

point of view, the range of fiber content recorded is advantageous, as fiber in food is essential to decreasing cholesterol and blood sugar. It has a high water-holding capacity during their passage through the digestive tract and constitutes roughage, facilitating gastrointestinal transit by reducing the transit time [36]. The calculated metabolizable energy values of defatted flours of *R. heudelottii* seed kernels presented in Table 1 ranged from 327.4 kcal/100 g (Akpi-Lak) to 339.2 kcal/100 g (Akpi-Vav). These values are close to those of biscuits made from compound flours (wheat flour + precooked taro meal + *R. heudelottii* oilcake) by Fombang et al. [37] with incorporation rates ranging from 0 to 35% of *R. heudelottii* oilcake. The differences observed in these samples could be due to the difference in protein and carbohydrate reported in proximate composition. Indeed, both carbohydrate (20.5–37.8%) and protein level (47.0–61.3%) were the highest contributors to calories from the determination of energy produced. Dry matter content of defatted kernels flours was found to vary between 92.3% and 95.6% of the fresh weight. These values are similar to those of Mezajoug [38]. Otherwise, the variation of dry matter implies that of water content in the samples. Low moisture content in foods limits microbial activities, while high moisture contents in food enhances microbial growth and hence food spoilage [39]. Thus, the low moisture content observed in this study (4.4–6.7%) is a desirable phenomenon. Another important aspect of the report is the statement that the lowest dry matter content (Akpi-Agb2: 92.3%, Akpi-Bon: 93.7%, Akpi-Div: 94.0%) had kernels that had undergone a very long cooking time, especially through pre-cooking and then cooking. This shows that, the longer the cooking time, the less the dry matter [38]. In contrast, dry matter content in Divo sample (Akpi-Div: 97.3%) would have increased with a relatively short cooking time and pre-cooking on a low heat. Thus, the long duration and the severity of cooking of *R. heudelottii* seeds certainly increased the absorption of water during cooking. Certainly, defatted flours from *R. heudelottii* seeds kernels are suitable for long-term storage, but each of them will not have the same shelf life. The ash content of samples ranged from 11.2% (Akpi-Vav) to 13.1% (Akpi-Div). These levels vary from one process to another and are high with ash values of 3.68%, 3.22%, and 3.56% reported for pigeon pea, lima bean, and lablab bean, respectively [40]. Ash content is an indicator for mineral elements [41]. Thus, from the result, it could be seen that Akpi-Div sample with 13.1% was the best in terms of mineral content. The minor ash variation could be due to the geographical origin of the samples, the climatic conditions, and the edaphic characteristics of soils [42]. Pomeranz and Clifto [43] recommends 1.5 to 3.5% of ash content in the seeds for animal feeds and human consumption. In this study, the ash content falls within this range, hence it can be recommended. For all samples, pH was slightly acid (6.1 to 7.1), while total acidity was low (1.9 to 4.5 meq/100 g). These values were close to that of rapeseed meal (pH 6.4) founded by Mayombo et al. [44]. The observed difference in the results may be related to the storage process, which is a function of the drying step [45]. Total acidity in the samples is consistent with the observations of Treche et al. [45]. These authors reported values between 2.9 and 4.6 mmol/MS 100 g in cassava flour in Congo (Central Africa) according to traditional processes of treatment of the tubers. The observed difference in titrable acidity is due to the drying time. For Treche et al. [45], the drying time has a significant influence on the acidity of the flour. A long drying time would reduce the titrable acidity of the flours. The determinations of titrable acidity, pH, and oxalic acid are of interest because of their alleged adverse effect on mineral bioavailability.

Secondly, we evaluated some minerals of defatted flours of *R. heudelottii* in order to assess the nutritional quality of the ash fraction. Minerals are essential nutrients and may help many body metabolic functions. Table 2 shows the concentration of mineral elements of defatted kernels flours of *R. heudelottii* for the various locations. Overall, the mineral and trace element contents differed significantly between samples. The mineral content of plants varies, generally, from soil types, whether fertilizer is ingested or not [46]. The results showed that defatted kernel flours of *R. heudelottii* are a good source of dietary minerals. Among the microelements, the predominant minerals were magnesium (12.0–40.0%). The seed kernel samples of *R. heudelottii* collected in six producing areas of Cote d'Ivoire were found to be the richest in phosphorus (0.3–2.0%), calcium (0.3–0.6%), and potassium (0.1–0.4%), while iron contents (2.0–3.0%) were similar among the samples. The magnesium content found revealed



significant variability with respect to traditional extraction process. The content in the samples could be related to the longer or shorter cooking time [38]. In our samples, the magnesium content was similar to the amounts found in the soya flours [47]. Phosphorus (P) levels, present in small amounts in samples, are lower than phosphorus content in rapeseed meal [44]. These low contents are thought to be related to phytate-iron-phosphorus complex formation [44], especially in the pre-cooking and cooking steps, which influence the mineral content by duration and intensity. Minerals: calcium (Ca) and potassium (K) represent the lowest levels in defatted kernels flours from *R. heudelottii*. Calcium contents are significantly lower than those in rapeseed meal (830 mg/10 g) and soybean meal (340 mg/100 g) obtained by Sauvant et al. [48], while potassium contents are also lower than those revealed in oilcakes of the same matrix in Cameroon [38]. Variations in calcium content could be due to calcium intake or not from cooking water through the crack in the hull. According to Lestradet and Machinot [49], boiling heavily increases the calcium intake during cooking. The magnitude of the difference depends on the temperature and the duration of treatment. It should be noted that chlorine and zinc were not detected in our samples.

**Table 2.** Mineral composition of defatted flours from *R. heudelottii* seeds kernels.

Defatted Kernel Flour	Mineral Elements						
	Mg (%)	P (%)	Ca (%)	K (%)	Fe (%)	Zn (%)	Cl (%)
Akpi-Agb1	19.0 ± 0.0 <sup>a,b</sup>	2.0 ± 0.0 <sup>a</sup>	0.6 ± 0.0 <sup>a</sup>	0.2 ± 0.0 <sup>a,b</sup>	3.0 ± 0.0 <sup>b</sup>	n.d.	n.d.
Akpi-Agb2	30.0 ± 0.0 <sup>b</sup>	1.0 ± 0.0 <sup>a</sup>	0.5 ± 0.0 <sup>b</sup>	0.3 ± 0.0 <sup>c</sup>	2.0 ± 0.0 <sup>a</sup>	n.d.	n.d.
Akpi-Div	20.0 ± 0.0 <sup>a,b</sup>	0.4 ± 0.0 <sup>b</sup>	0.4 ± 0.0 <sup>c,d</sup>	0.1 ± 0.0 <sup>a</sup>	2.1 ± 0.0 <sup>a</sup>	n.d.	n.d.
Akpi-Lak	12.0 ± 0.0 <sup>a</sup>	0.3 ± 0.0 <sup>b</sup>	0.3 ± 0.0 <sup>c</sup>	0.2 ± 0.0 <sup>a,b</sup>	2.0 ± 0.0 <sup>a</sup>	n.d.	n.d.
Akpi-Bon	30.0 ± 0.0 <sup>a,b</sup>	0.4 ± 0.0 <sup>c</sup>	0.4 ± 0.0 <sup>d</sup>	0.4 ± 0.0 <sup>d</sup>	2.1 ± 0.0 <sup>a</sup>	n.d.	n.d.
Akpi-Vav	40.0 ± 0.0 <sup>c</sup>	1.0 ± 0.0 <sup>a</sup>	0.6 ± 0.0 <sup>a,b</sup>	0.2 ± 0.0 <sup>a,b</sup>	2.3 ± 0.0 <sup>a</sup>	n.d.	n.d.

Data are represented as Means ± SD ( $n = 3$ ). (<sup>a,b,c</sup>) 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 of Agboville1; Akpi-Agb2 = Akpi of Agboville2; Akpi-Vav = Akpi of Vavoua; Akpi-Div = Akpi of Divo; K: Potassium; P: Phosphorus; Ca: Calcium; Mg: Magnesium; Fe: Iron; Zn: Zinc; Cl: Chlorine. n.d.: no detected

The trace elements levels (iron) detected in kernels were moderate compared to the values (8 and 6 mg/day, respectively) recommended for human dietary allowance [50]. In this study, the extraction process methods of *R. heudelottii* seeds kernels did not considerably affect the determination of iron produced.

Finally, we evaluated some of the nutritive and antioxidant compounds of defatted flours from *R. heudelottii* kernels (Table 3). The result of the analysis revealed an appreciable amount of 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 oxalates and phytates. Indeed, there was high variability with respect to traditional extraction process. The polyphenols are the main dietary antioxidants among selected *R. heudelottii* seeds kernels. In our samples, the total polyphenols content ranges from 216.6 mg/100 g (Akpi-Bon) to 403.9 mg/100 g (Akpi-Agb1) and was low when compared to defatted flours from *R. heudelottii* kernels collected in local food spice market in Mbalmayo, Cameroon (940.00 mg/100 g). On the other hand, these amounts are higher than those found in the defatted flours particles fractions: >500  $\mu\text{m}$  (170.01 mg/100 g), 400–500  $\mu\text{m}$  (210.0 mg/100 g), and <160  $\mu\text{m}$  (80.05 mg/100 g) of *R. heudelottii* kernels [6]. Following the example of previous studies that tested fruits from tropical regions for their polyphenol contents [51], all defatted flours samples of *R. heudelottii* evaluated in this study can be categorized as having a low concentration of phenolic compounds (<500 mg/100 g); consequently, they are a low source of phenolic compounds. As postulated by Mehinagic et al. [52], 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. [53], the concentration of flavonoids was high but lower compared to the amounts found in soya [54]. Defatted flours from *R. heudelottii* kernels collected in Vavoua (4.6 mg/100 g) had the highest levels of flavonoids followed by those collected in Divo (3.1 mg/100 g), Agboville2 (2.7 mg/100 g), Agboville1 (1.8 mg/100 g), Bondoukou (1.1 mg/100 g), and Lakota (1.0 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 was also found by Bolanho and Beléia [54]. It is now common knowledge that various post-harvest traditional technologies can reduce and/or destroy several nutritional factors [55]. Flavonoids have a varied biological activity, which includes their ability to eliminate biological radicals and superoxide anions radicals. Flavonoids therapy can also be used in the treatment of some disorders due to its anti-inflammatory, anti-angionic, and anti-allergic effects, as well as its analgesic and antioxidant properties [56]. On the other hand, it has been observed that the defatted flours from *R. heudelottii* kernels used in this study contained anti-nutrients, with values ranging from 714.7 mg/100 g (Akpi-Vav) to 972.9 mg/100 g (Akpi-Agb1) for oxalates, 16.4 (Akpi-Agb1) to 41.8 mg/100 g (Akpi-Lak) for tannins, and 52.2 (Akpi-Div) to 71.4 (Akpi-Vav) for phytates.

**Table 3.** Bioactive compounds content of defatted flours from *R. heudelottii* seeds kernels.

Defatted Kernel Flour	Bioactive Compounds				
	Polyphenols (mg/100g)	Phytates (mg/100g)	Flavonoids (mg/100g)	Tannins (mg/100g)	Oxalate (mg/100g)
Akpi-Agb1	403.9 ± 0.6 <sup>a</sup>	62.3 ± 0.1 <sup>a</sup>	1.8 ± 0.0 <sup>a,b</sup>	16.4 ± 0.0 <sup>a</sup>	972.9 ± 0.0 <sup>a</sup>
Akpi-Agb2	346.4 ± 0.1 <sup>a,b</sup>	52.3 ± 0.3 <sup>a</sup>	2.7 ± 0.0 <sup>a,b</sup>	23.9 ± 0.0 <sup>b</sup>	972.3 ± 0.0 <sup>a</sup>
Akpi-Div	347.4 ± 1.2 <sup>a,b</sup>	52.2 ± 0.1 <sup>a</sup>	3.1 ± 0.0 <sup>b</sup>	21.8 ± 0.0 <sup>a,b</sup>	973.6 ± 0.0 <sup>a</sup>
Akpi-Lak	285.2 ± 0.1 <sup>b</sup>	61.9 ± 0.1 <sup>a</sup>	1.0 ± 0.0 <sup>a</sup>	41.8 ± 0.1 <sup>c</sup>	881.1 ± 0.0 <sup>a</sup>
Akpi-Bon	216.6 ± 0.2 <sup>c</sup>	63.3 ± 0.0 <sup>a</sup>	1.1 ± 0.0 <sup>a</sup>	18.5 ± 0.1 <sup>a,b</sup>	908.1 ± 0.0 <sup>a</sup>
Akpi-Vav	348.4 ± 0.0 <sup>a,b</sup>	71.4 ± 0.1 <sup>a</sup>	4.6 ± 0.0 <sup>c</sup>	22.0 ± 0.0 <sup>a,b</sup>	714.7 ± 0.0 <sup>a</sup>

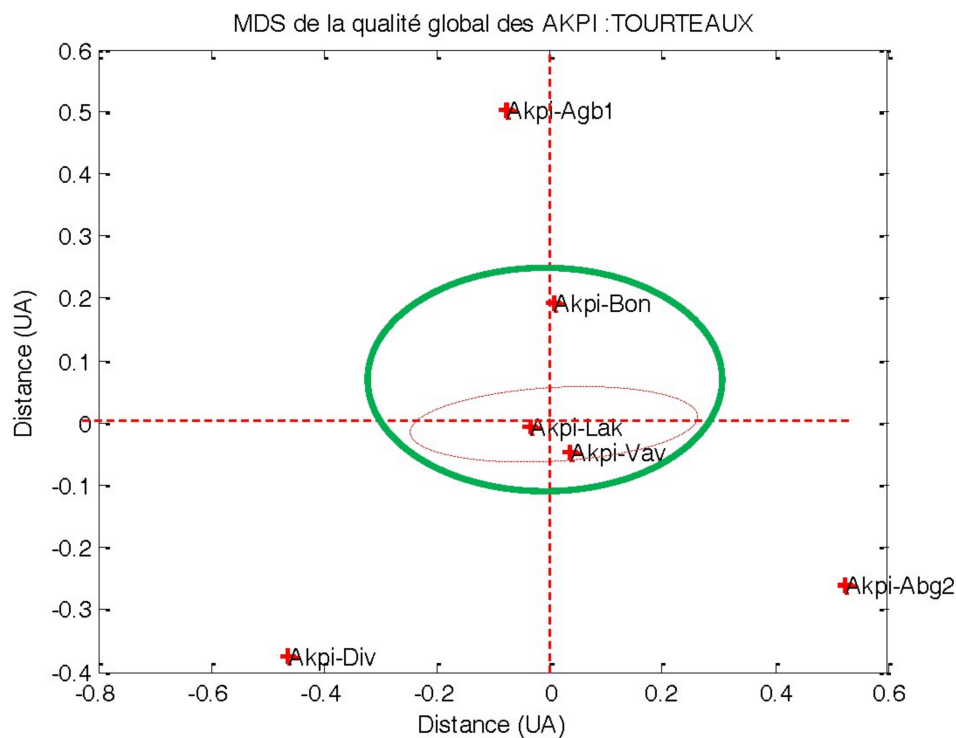
Data are represented as Means ± SD ( $n = 3$ ). (<sup>a,b,c</sup>) The values within a column with different superscript letters are significantly ( $p < 0.05$ ) different according to Duncan's test. Akpi-Lak = Akpi of Lakota; Akpi-Bon = Akpi of Bondoukou; Akpi-Agb1 = Akpi of Agboville1; Akpi-Agb2 = Akpi of Agboville2; Akpi-Vav = Akpi of Vavoua; Akpi-Div = Akpi of Divo.

Their presence in the samples has a deleterious effect in the areas of human nutrition and food production. For example, oxalate is a chelating agent that binds calcium very effectively [41]. As mentioned earlier, the defatted flours from *R. heudelottii* seed kernels are a good source of dietary minerals, although in this study their oxalate and phytate contents were equally high. The oxalate amounts found in the kernel's defatted flour are similar to other plant food rich in oxalate [57] but are distinctly lower compared to the content found by Mezajoug and Tchiégang [6], which presented more than 2000 mg/100 g of oxalates in defatted flours from *R. heudelottii* seeds kernels. In all cases, it is now known that vegetables-based diets with high oxalate content can lead to an acute loss of metabolic calcium (hypocalcemia) [41]. The phytate amount of defatted flours from *R. heudelottii* kernels is very high compared to 17.0–18.1 mg/100 g found by Mezajoug and Tchiégang [6] for unsieved defatted flours of *R. heudelottii* kernels. The highest concentrations of phytate could be disadvantageous to the health status of consumers. Indeed, phytates are anti-nutrients that chelate divalent cations such as  $Zn^{2+}$ ,  $Fe^{2+}$ ,  $Fe^{3+}$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Mn^{2+}$ , and  $Cu^{2+}$ , thereby reducing their bioavailability. It is well documented that tannins negatively affect protein digestibility by reducing the bioavailability of iron and calcium intake, as well as carbohydrates, which lowers the energy value of dietary intakes [58]. However, its anti-nutritional effects are a function of their chemical structure and dosage [59]. In the case of our samples, tannin level was found to be high in comparison with tannic acid found in some literatures [6,38,53]. The differences between them probably indicate a beneficial effect on total tannin content due to cooking process. This fact is also corroborated by those mentioned by Mezajoug [38]. This author showed that long cooking times (90–120 min) reduced the tannin content

significantly. Because our sampling is subject to many parameters such as geographical area of production, cultivation cycle, and climatic conditions, consistent interpretations of results may be hindered. Thus, multidimensional scaling (MDS) was applied to the physicochemical parameters for better conclusions.

#### MDS Applied to Defatted Flours from *R. heudelotii* Sample Data

By performing principal coordinate analysis, the MDS plot presented in Figure 3 was obtained.



Akpi-Lak = Akpi of Lakota; Akpi-Bon = Akpi of Bondoukou; Akpi-Agb1 = Akpi of Agboville1; Akpi-Agb2 = Akpi of Agboville2; Akpi-Vav = Akpi of Vavoua; Akpi-Div = Akpi of Divo

**Figure 3.** Multidimensional scaling (MDS) plot analysis conducted with the proximate and element composition data characterizing defatted flours from *R. heudelotii* seeds kernels.

The dimensions (Distance UA in Figure 3) are factors with real meaning that make it possible to explain the differences among groups [60]. The MDS plot (Figure 3) shows that the defatted flours from *R. heudelotii* samples were clearly classified into Bondoukou-Lakota-Vavoua diagram (Akpi-Lak; Akpi-Bon; Akpi-Vav, code sample) and individual samples (Agboville1 diagram, Divo diagram and Agboville2 diagram). Considering the only group composed of the defatted flours from *R. heudelotii* grown in Bondoukou, Lakota, and Vavoua, it can be observed from the multidimensional positioning graph that the production processes of Bondoukou, Lakota, and Vavoua show strong similarities in separate quadrants. The similarity attributed to phosphorus is observed in similar trends (Table 2), especially for Lakota and Vavoua samples. This similarity is also expressed by the F1 distances expressed to the level in dry matter (0.941188), moisture (0.774839), ash (0.716755), proteins (0.854214), total carbohydrates (0.895931), energy value (0.930116), polyphenols (0.787436), and flavonoids (0.762606). Characteristic distances of similarity agree with data in Tables 1 and 2. From these data, the approximation is more evident between the Lakota and Bondoukou samples on the graph (Figure 3). They have 94.0 and 93.7% dry matter, respectively, and 12.0% and 12.2% ash, respectively, just to mention a few. These data could also be attributed to the fact that the production

systems of both Lakota and Bondoukou cakes have similar stages, namely pre-cooking stage followed by the renewal of cooking water before the cooking stage, while in Vavoua these steps are not practiced. These observations suggest that the extraction process of kernels from Bondoukou, Lakota, and Vavoua are similar reading from the diagram. Agboville1 samples differed from the others by the MDS output dimensions in terms of dry matter, ash, protein, total carbohydrates, fiber, pH, acidity, and caloric energy. In effect, the difference in the position of this sample on the graph would be related to the average values obtained for quoted parameters. Most of these parameters are the singular values for defatted flours from Agboville1. This sample also differs by the MDS output dimensions in level of polyphenols, phytates, flavonoids, tannins, and oxalates. The dissimilarity with the other samples is shown by the result in Table 3. This sample has the highest content of polyphenols (403.9 mg/100 g) and the lowest tannin content (16.4 mg/100 g), as well as singular contents in phytates (62.3 mg/100 g) and flavonoids (1.8 mg/100 g). The uniqueness of these findings in particular could be justified by the kernels production system. This system includes the step of preliminary drying of the seeds after cooking before the extraction of kernels. This step may influence the contents of the different substances. As for Divo, they differ from the other samples in dry matter, protein, total carbohydrate, and pH. These differences are shown by the average content of these parameters. Divo samples have the highest rate of dry matter (95.6%). The differences are also confirmed by the analysis of the variance, which indicates significant differences between the parameters ( $p \leq 0.05$ ) (Table 1). Also, the MDS output distances recorded for polyphenols, phytates, flavonoids, tannins, and oxalates showed how different Divo sample was from the others. The dissimilarities in these parameters, as observed in Table 3, could be particularly attributed to the long pre-cooking time (all night) in the production process of *R. heudelottii* kernels. The difference in the result therefore could be linked to the pre-cooking. The uniqueness of Agboville2 resides in the parameters such as dry matter, proteins, total carbohydrates, pH, polyphenols, phytates, flavonoids, and oxalates. This feature is characterized by the dimensions of MDS output obtained for each of the parameters; the dimensions included between 0.8 and 0.9. The average dry matter content (92.3%) illustrates the difference between this sample and the others (Tables 1 and 3). Agboville2 sample had the lowest dry matter content. The dissimilarity in these results could be due to the direct cooking of *R. heudelottii* fruits after their harvest (Figure 2), thus replacing the fermentation stage of the fruits. This cooking of fruits could cause significant changes to the different levels of biochemical parameters of the defatted flours from *R. heudelottii* seeds kernels. Variance analysis confirms these significant differences between the contents for these quoted parameters ( $p \leq 0.05$ ).

#### 4. Conclusions

The results in the present study reaffirm that defatted flours from *R. heudelottii* seeds kernels have high nutrient composition and calorie value, most especially in terms of carbohydrate (total dietary fiber) and protein. Among the mineral elements investigated (P; Ca; K; Fe; Zn; Cl), magnesium was the predominant mineral, while polyphenols and oxalates were the main phytochemical compounds identified. From these results, the kernel extraction techniques employed may influence the physico-chemical composition and antioxidant potential of defatted flours of *R. heudelottii* seed kernels, particularly the contents of crude protein, total dietary fiber, polyphenols, flavonoids, tannins, and ash (Mg, P, Ca, and K). The application of the MDS technique made possible the selection of chemical compounds that are responsible for the main differences among samples. Thus, the MDS technique made possible the selection of four main extraction systems: the Bondoukou-Lakota-Vavoua diagram, the Agboville1 diagram, the Divo diagram, and the Agboville2 diagram. These results provide useful indications of the effect of the traditional extraction process on the physico-chemical and antioxidant compounds of defatted kernels flours from *R. heudelottii*.

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