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Effects of production site and coat colour on the physicochemical characteristics of cowpea (*Vigna unguiculata* L. Walp) seeds in Côte d'Ivoire

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Abstract
Cowpea seeds are produced in many sites and consumed in Côte d'Ivoire. These seeds varied widely in coat colour. The present study was carried out to check the influence of some factors (production site and coat colour) on the physicochemical characteristics of cowpea seeds. For this purpose red seed samples and white ones were drawn from three production sites. The physicochemical characteristics were determined following standard methods while the influence of factors was checked out via chemometric methods. The results showed that cowpea seeds are rich in carbohydrates (63.76 ± 0.85%) and proteins (21.29 ± 0.94%). Its characteristics were significantly (p<0.05) affected by seed coat colour and production sites. The effects of their interaction were also shown to be significant. In addition, the significant (p<0.05) effects of coat colour, production site and their interaction on physicochemical properties of cowpea seeds are responsible of differences observed between samples analysed. Cowpea samples from *Marahoue* site which seed coat is white recorded the highest protein content while the red ones collected in *Tonkpi* site recorded highest value of carbohydrates. These samples could be used to fight against nutritional diseases due to protein and carbohydrate deficiencies.

Keywords: Cowpea, influence, production site, coat colour, seed weight, chemometric methods

1. Introduction
Cowpea (*Vigna unguiculata* (L) Walp) is one of the most ancient crops. It is mainly cultivated for its seeds which constitute an important source of nutrients. Besides, these seeds are the most economically valuable plant part of cowpea and are well-known due to their ascribed nutritional and medicinal properties (Zia-Ul-Haq *et al*., 2013) [1]. Cowpea is an important source of plant protein for people who cannot afford proteins from animal sources such as meat and fish (Hamid *et al*., 2016) [2].

In many parts of West Africa, cowpea seeds are well-known and consumed. About of 36,000 tons per year of West African productions were from Côte d'Ivoire (Ivory Coast) (FAOSTAT, 2014) in which the main production sites are localized in the centre and northern. In this country, edible cowpea seeds are consumed as boiled seeds alone or can be processed into various products such as flours, fried cowpea paste, etc. The seeds are also consumed in combination with other foods such as rice, maize and plantain (Inobeme *et al*., 2014) [4]. Processing levels included home-based food processing and industrial level (Fasoyiro *et al*., 2012) [5]. Due to its nutrient and functional benefits cowpea has gained industrial importance for being used as a potential ingredient in food formulations (Hamid *et al*., 2016) [2]. But, the potential of cowpea seeds to be processed into value-added products, would be influenced by their physical and chemical properties (Henshaw, 2008) [6]. It is therefore necessary to investigate the proximate composition of seeds.

In addition, it is well-known that cowpea seeds are characterised by a wide variation in coat colour (Odindo, 2007) [7]. Thus, seed coat, which is either smooth or wrinkled, can be white, cream, green, buff, red, brown, or black, etc. These colour differences have implications for cowpea products characteristics (Chinma *et al*., 2008) [8]. In the other hand, the seed coat colour is an important property which influence consumer acceptance of cowpea varieties (Henshaw, 2008) [6]. The brown coloured seeds are preferred to cream/white for cooking by boiling because they provide a sensory appeal by their colour. While the cream/white coloured varieties are mainly used in products requiring dehulling (removal of the seed coat) such as cowpea paste

and flour. In Côte d'Ivoire, data on the influence of seed coat colour on cowpea physicochemical characteristics are not well-documented. Then, the effect of seed coat colour on the physicochemical parameters of cowpea must be investigated.
Taking into account interesting observations made by Liu (1997) [9] who reported that the chemical composition of legumes varied depending on variety, species and region, the effect of sampling regions or production sites on the physicochemical characteristics of cowpea must also be investigated.
The present study was carried out to provide data on cowpea (*Vigna unguiculata*) seeds and sought to understand the effects of production site and coat colour on physicochemical characteristics of cowpea seeds. The effects of these factors were checked out via chemometric methods. The results obtained will serve as a guide for future research.

2-Material and methods

2.1 Raw materials

Cowpea seeds used in this study were obtained from three production sites (i.e., *Marahoue*, *Tonkpi* and *Poro*) of Côte d'Ivoire in which cowpea is widely consumed and available. In each production site, three white samples of cowpea and three red ones were purchased from markets. It is necessary to underline that samples were ranked visually, on the basis of seed coat colour into white or red cowpea. In total, eighteen samples of cowpea seeds were collected.

2.2. Physicochemical analyses

2.2.1 100-seed weight

The seed weight was determined by weighing hundred randomly selected seeds of each sample (AOAC, 2000) [10] using Denver Instruments apparatus (N° TP-214). Average of three determinations was reported.

2.2.2 Chemical analyses

The following analyses were conducted to characterize the cowpea seeds. Moisture, ash, proteins and lipids contents were evaluated using BIPEA (1976) [11] methods. Total carbohydrate contents were determined by difference. Caloric energy was calculated according to the Atwater general factor system (FAO, 2003) [12]. The system uses a single factor for each of the energy-yielding substrates (protein, fat, carbohydrate) regardless of the food in which it is found. The energy values are 4.0 kcal/g for protein, 9.0 kcal/g for fat and 4.0 kcal/g for carbohydrates.

2.3 Chemometric methods

Data recorded from the physicochemical characterization were subjected to Statistical analyses. Cluster analysis and multidimensional analysis of variance (MANOVA) were done. Whereas, the former consists in grouping identical individuals into sets, the later deals with comparing different means in order to identify if they are different or equal. In this study, cluster analysis was based on Ward's method as agglomerate one. The square Euclidean distance was the chosen metric as recommended by Johnson and Wichern (2007) [13]. The quality of the typology was evaluated by the proportion (%) of total sum of squares explained. This proportion is calculated using agglomeration schedule according to Tenenhaus (2011) [14]. The MANOVA is a generalization of analysis of variance (ANOVA) method to one or several factors (qualitative variables) in which two or several dependent variables are measured simultaneously. It enables to examine the main effects and factors interaction. Analysis of variance (ANOVA) was also done. If necessary, Tukey HSD multiple comparison tests were done to determine significant differences at 5% probability between means. In addition, Principal Components Analysis (PCA) was performed when needed. The PCA is a linear dimensionality reduction technique, which identifies orthogonal directions of maximum variance in the original data, and projects the data into a lower dimensionality space formed of a sub-set of the highest-variance components. The above analyses were performed using Statistica 7.1. Software package.

3. Results

3.1 Physicochemical characteristics of cowpea seeds

The physicochemical parameters of cowpea samples were determined. The results are presented in Table 1. As shown in this table, the cowpea seeds weights were between 8.55 and 27.38 g with an average of 17.41 ± 5.08 g. An average moisture value of about 7.64 ±0.86% were recorded. The ash, fat and total carbohydrate contents of cowpea seeds were 3.89 ± 0.39%, 3.40 ± 0.60% and 63.76 ± 0.85%, respectively. They contained moreover proteins (21.29 ± 0.94%). The cowpea seed was rich in carbohydrates and proteins. In addition, consumption of cowpea seeds provides an average energy of 370.90 ± 3.56 kcal /100 g. Besides, some physicochemical parameters of seed samples have relative standard deviation greater than 15%. These included fat content and 100-seed weight with respective values of 17.66 and 29.17% (Table 1).

Table 1: Physicochemical characteristics of cowpea seed samples.

Parameters	N	Means	Minimum	Maximum	SD	RSD
100-seed weight (g)	18	17.41	8.55	27.38	5.08	29.17
Moisture (%)	18	7.64	6.51	9.21	0.86	11.34
Ash (%)	18	3.89	3.03	4.21	0.39	10.07
Fat (%)	18	3.40	2.18	4.13	0.60	17.66
Carbohydrates (%)	18	63.76	62.73	65.39	0.85	1.34
Proteins (%)	18	21.29	20.61	23.35	0.94	4.45
Energy (kcal/100 g)	18	370.90	365.02	375.78	3.56	0.96

SD: Standard deviation; RSD: Relative standard deviation; N: Number of observations

3.2 Identification of differences between cowpea seed samples

Figure 1 presents the result of cluster analysis on cowpea samples. The analysis of the hierarchical tree reveals the existence of physicochemical differences between seed samples. Indeed, when the tree is cut to a distance of 100 (arbitrary unit), four classes can be distinguished:

- A class formed by seeds with white coat coming from *Marahoue* site;
- A second class highly composite that grouped red seeds from *Tonpki* and *Marahoue* sites and some white seeds collected in *Poro* site;

- A third class, composed by the seeds with red coat colour from *Tonkpi* site and a white seeds sample from the production site of *Poro*;
- A fourth group formed by white seeds from *Tonkpi* site.

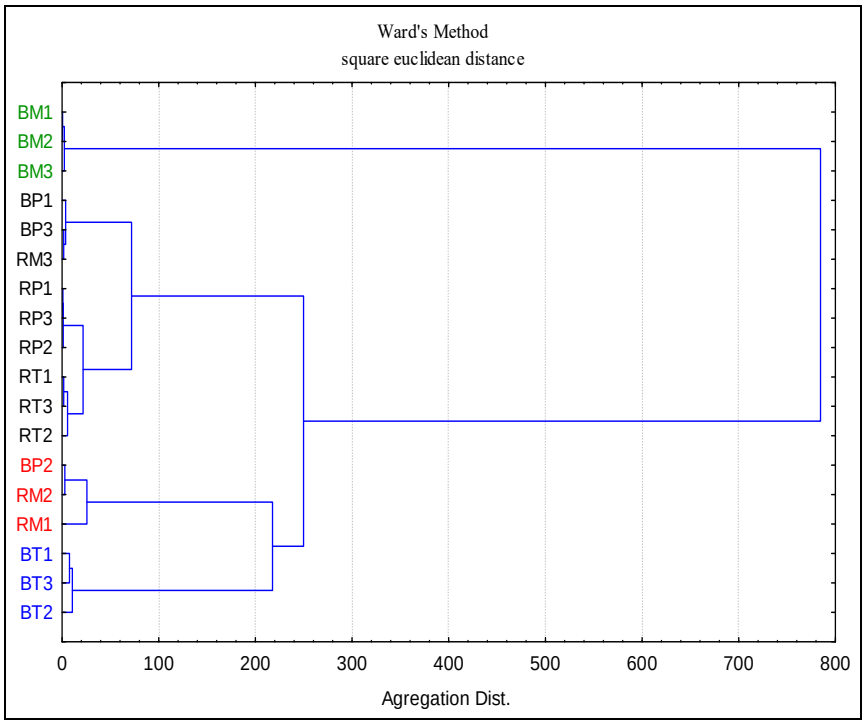

Fig 1: Hierarchical tree of cowpea samples analysed.

The typology in four classes takes in account more than 88.82% of differentiations between cowpea samples (Table 2). The distribution of the cowpea samples in a factorial plan (F1-F2) is presented on figure 2. As shown, many groups of cowpea seeds were observed. In this factorial plan, the first component was characterised by variables such as proteins, energy, fat and 100-seed weight while the second one was determined by ash and moisture contents (Figure 3).

Table 2: Quality of typology.

Number of group	Total sum of Squares	Sum of squares within groups of the typology	Proportion (%) of total sum of squares explained
2	704.794	312.528	55.65
3	704.794	187.614	73.37
4	704.794	78.789	88.82

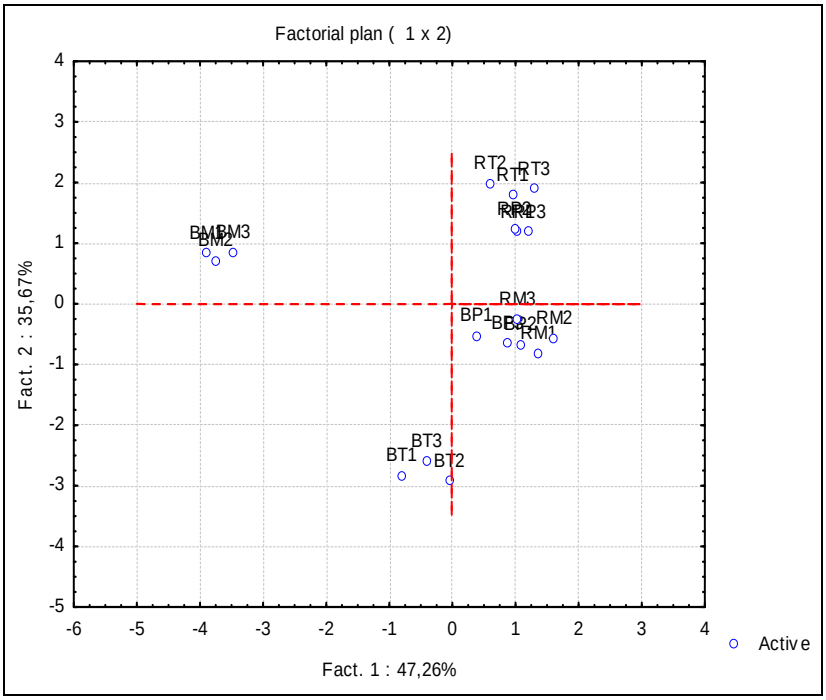

Fig 2: ACP plot on cowpea seeds samples analysed (F1-F2).

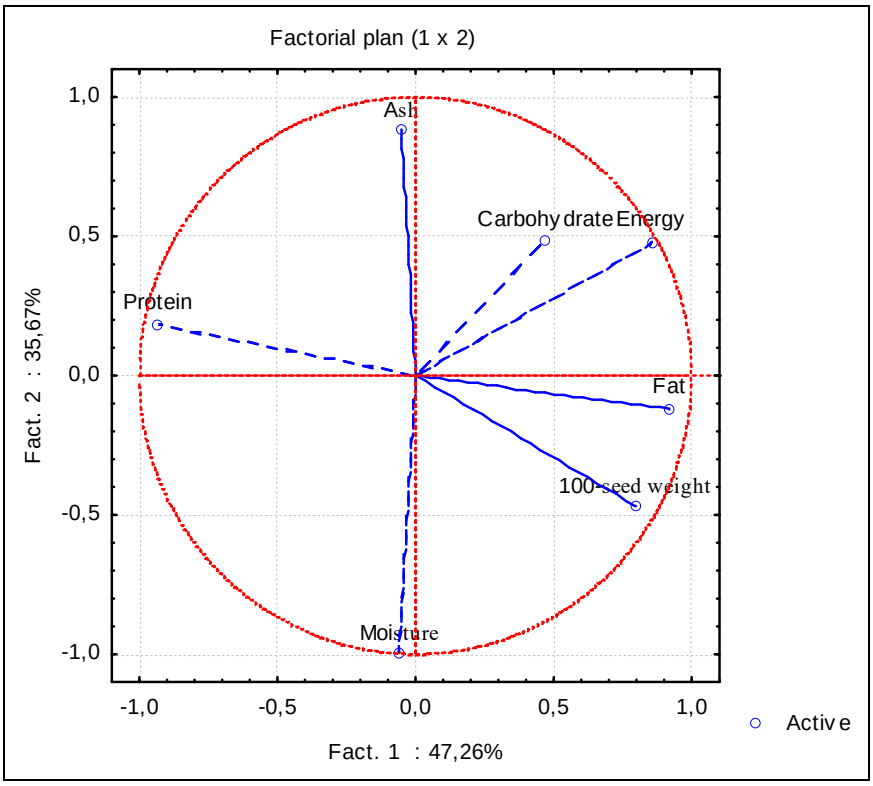

Fig 3: Circle of Correlation between seed variables.

3.3 Influence of production site and coat colour on physicochemical characteristics of cowpea seeds

The influence of factors is firstly checked out using MANOVA procedure. Table 3 presents the results of multidimensional analysis of variance on the physicochemical parameters of cowpea seeds. As shown in this table, the main effects of coat colour and production site in one hand and the effects of interaction between the both factors in the other hand, affected significantly (p<0.05) the whole parameters analysed. The effects of seed colour and production sites on each physicochemical parameter of cowpea were determined via factorial ANOVA. Table 4 presents the results. As shown, the effects of seed colour and production site were significant (at p<0.05) on moisture, ash, fat, carbohydrates, proteins contents and energy value. On the 100-seed weight, only the effect of seed colour is significant (at p<0.05).

Table 3: MANOVA table from physicochemical characteristics of cowpea seeds.

Effects	Test	Value	F	Effect dl	Error dl	P
Colour	Wilk	0.001635	977	5	8	0.00
Production sites	Wilk	0.000033	279	10	16	0.00
colour*production sites	Wilk	0.000035	268	10	16	0.00

Effect is significant at p<0.05

Table 4: Results of factorial analysis of variance on cowpea physicochemical characteristics.

Parameters	Effects	Sum of square	Degree of freedom	Mean of square	F	P
100-seed weight	Colour	32.428	1	32.428	8.602	0.01
	Production sites	26.724	2	13.362	3.544	0.06
	Colour*production sites	334.503	2	167.251	44.366	0.00
Moisture	Colour	4.580	1	4.580	4580	0.00
	Production sites	0.470	2	0.235	235	0.00
	Colour*production sites	7.730	2	3.865	3865	0.00
Ash	Colour	0.4544	1	0.4544	659.6	0.00
	Production sites	1.0167	2	0.5084	738.0	0.00
	Colour*production sites	1.1323	2	0.5662	821.9	0.00
Fat	Colour	1.0561	1	1.0561	35.545	0.00
	Production sites	1.5445	2	0.7723	25.993	0.00
	Colour*production sites	3.2098	2	1.6049	54.017	0.00
Carbohydrates	Colour	6.50	1	6.50	317	0.00
	Production sites	4.79	2	2.40	117	0.00
	Colour*production sites	1.01	2	0.50	25	0.00
Proteins	Colour	4.46	1	4.46	857	0.00
	Production sites	5.67	2	2.83	544	0.00
	Colour*production sites	5.13	2	2.56	493	0.00
Energy	Colour	121	1	121	146	0.00
	Production sites	41	2	20	25	0.00
	Colour*production sites	45	2	22	27	0.00

Effect is significant at p<0.05.

3.4 Comparison between the physicochemical characteristics of cowpea seeds from three production sites

Table 5 presents the physicochemical characteristics of cowpea seeds from three production sites. The analysis showed that the moisture and fat contents varied respectively from 7.49±0.50% (in *Poro* site) to 7.86±1.45% (*Tonkpi* site) and from 3.13±0.89% (*Marahoue*) to 3.81±0.24% (*Poro*). It was observed that there was no significant difference (p>0.05) between moisture content of cowpea seeds from these regions. In the same mood, energy value and fat content of cowpea did not varied significantly from a site to another. In addition, whatever is the production site, the 100-seed weight did not varied significantly (16.14±8.53 – 19.05±2.14 g). In the other hand, there was a significant difference (p<0.05) between the values of ash (Table 5). The lowest value was recorded in *Tonkpi* site (3.55±0.53%) while the highest one, reached 4.06±0.12% in *Poro* site. The values of carbohydrates were between 63.15±0.31% and 64.42±0.79% recorded in *Marahoue* and *Tonkpi* sites respectively. This variation is revealed to be statistically significant (p<0.05). The seeds contained also proteins, which values varied from 20.88±0.07 (*Tonkpi* site) to 22.08±1.35% (*Marahoue* site). There was a significant difference (p<0.05) between protein contents. The highest value was recorded in *Marahoue* site while the lowest one was 20.88±0.07.

Table 5: Physicochemical characteristics of cowpea seeds from three production sites.

	Production sites		
Parameters	***Marahoue***	***Poro***	***Tonkpi***
100-seed weight (g)	16.14±8.53^{a}	19.05±2.14^{a}	17.04±2.24^{a}
Moisture (%)	7.56±0.30^{a}	7.49±0.50^{a}	7.86±1.45^{a}
Ash (%)	4.05±0.13^{a}	4.06±0.12^{a}	3.55±0.53^{b}
Fat (%)	3.13±0.89^{a}	3.81±0.24^{a}	3.27±0.24^{a}
Carbohydrates (%)	63.15±0.31^{a}	63.72±0.90^{ac}	64.42±0.79^{bc}
Proteins (%)	22.08±1.35^{a}	20.90±0.30^{b}	20.88±0.07^{b}
Energy (kcal/100 g)	369.18±3.85^{a}	372.84±0.71^{a}	370.69±4.44^{a}

Tabulated values are means of six determinations ± Standard Deviation (SD)

Values with different letters in each row are significantly different (p<0.05)

3.5 Physicochemical characteristics of cowpea seeds from two coat colour

Table 6: Physicochemical characteristics of cowpea seeds from two coat colour

Parameters	White cowpea	Red cowpea
100-seed weight (g)	16.07±5.71^{a}	18.75±4.25^{a}
Moisture (%)	8.14±0.83^{a}	7.13±0.56^{b}
Ash (%)	3.73±0.50^{a}	4.05±0.10a
Fat (%)	3.16±0.74^{a}	3.65±0.28^{a}
Carbohydrates (%)	63.16±0.42^{a}	64.36±0.75^{b}
Proteins (%)	21.78±1.15^{a}	20.79±0.14^{b}
Energy (kcal/100 g)	368.31±3.25^{a}	373.5±1.14^{b}

Tabulated values are means of nine determinations ± Standard Deviation (SD)

Values with different letters in each row are significantly different (p<0.05)

Table 6 presents the physicochemical characteristics of cowpea seeds depending on coat colour. The analysis revealed that whatever is the seed coat colour, the parameters such as 100-seed weight, ash and fat contents did not varied significantly (p>0.05). In contrary, moisture, carbohydrates and proteins contents of cowpea varied from a seed coat colour to another. The red cowpea recorded the highest values of ash (4.05±0.10%), carbohydrates (64.36±0.75%) and energy (373.5±1.14 kcal/100 g). According to moisture and proteins contents, the white cowpea recorded the highest values about 8.14±0.83% and 21.78±1.15%, respectively.

4. Discussion

The physicochemical characterization revealed that cowpea seeds were rich in carbohydrates and proteins. This confirms the fact that cowpea is a legume. The protein content of cowpea seeds was found to be 24.5% wet basis (Frota *et al*., 2008) [15]. In this study, the observed values were below. Hamid *et al*. (2015) [16] found values of crude protein in the range of 19.6–23.23%. Similar values were recorded in this survey. In addition, cowpea seed had high carbohydrate content. These results are in agreement with earlier report (Koko *et al*., 2016) [17]. These authors found average values of 65.81±0.02% in white cultivar black-eyed from Côte d'Ivoire. The cowpea seeds are energizing foods due certainly to their high carbohydrate and protein contents. In this study, the values of caloric energy were close to those recorded in cowpea local varieties in Nigeria, ranging from 337.57±1.30 to 360.67±2.43 kcal/100 g (Chinma *et al*., 2008) [8]. The low moisture content of seeds is an indication of a good shelf life if packaged and stored (Hayma, 2004) [18]. In addition, cowpea seeds have low fat content. It is well-known that these seeds are low-fatty foods (Famata *et al*. 2013; Hamid *et al*., 2015) [19, 16]. The relatively high ash content indicates that the cowpea varieties could be important sources of minerals for consumers. The seed weight of cowpea variety could be a useful criterion for determining suitability for a particular end-use application (Henshaw, 2008) [6]. In this study, the 100-seed weight varied widely with an average value of 17.41 ± 5.08 g. That leads to a RSD greater than 15%. It suggests that differences seem existed between cowpea samples analysed. This idea is confirmed by the fact that some parameters (e.g., fat content and 100-seed weight) have high RSD (>15%). Moreover, the analysis of the hierarchical tree obtained by cluster analysis reveals the existence of physicochemical differences between cowpea samples. Indeed, when the tree is cut to a distance of 100 (arbitrary unit), more than 88.82% of the differentiations between cowpea samples are explained and four classes can be distinguished. The high quality of typology in four classes indicates that there are significant differences between samples analysed.

The distribution of cowpea samples in classes could be explained by the influence of some factors on the physicochemical parameters. These factors might include production site and seed coat colour. The influence of such factors is checked out using MANOVA procedure. The results showed that the effects of seed coat colour and production site on the physicochemical parameters of seeds are significant (P<0.05). Thus, these factors are responsible of variations noted on seed characteristics. The effects of seed coat colour could be explained by the existence of specific characteristics belonging to a cultivar. According to Hamid *et al*. (2015) [16], a physicochemical parameter as the polyphenol content varied widely in seed coat colour. They reported that the polyphenol content was found significantly higher for Red cultivar (0.27%) than the Black cultivar (0.24%). In addition, it is well-known that the seed coat colour influenced the colour of flour which is important as per its industrial application (Hamid *et al*., 2015) [16].

The influence of production site could be explained by environmental conditions and soil characteristics. Indeed, Temperature, photoperiod, water deficits and soil fertility are known for their effects on seed mass, number and quality (Bewley and Black, 1994; Dornbos, 1995; Wulf, 1995) [20, 21, 22]. Climatic changes and agronomic practices may also affect seed composition (Thomas *et al*., 2003; Henshaw, 2008) [23, 6]. In addition, the results of factorial ANOVA have clearly shown that, both production site and seed coat colour influenced significantly all the physicochemical parameters analysed except the 100-seed weight. This implies that the physicochemical characteristics of cowpea seeds varied from a sample to another depending on the coat colour and the production site. The effects of cultivars and regions on changes in physicochemical characteristics of products have already been reported (Koko *et al*., 2010) [24]. Besides, seed weight is largely a function of seed components (Singh *et al*., 1968) [25]. In this study, the main factor that influence its value is the seed coat colour. In the same mood, the interaction between colour of seed coat and production site affects the 100-seed weight. Thus, Ogle *et al*. (1987) [26] classified cowpea varieties into size categories based on their 100-seed weight. Varieties with seed weight between 10-15 g are described as small; 15.1-20 g are medium size-seed while large seed have 20.1-25 g. Seed weight over 25 g are described as very large seeds. The average value of 17.41 ± 5.08 g recorded in this study is below 25 g and revealed that the seed samples analysed were not very large. The seed weight is the most discriminating physical property among the cowpea varieties studied (RSD=29.17%). This property may become an important criterion for selecting cowpea variety for processing into different end products (Henshaw, 2008) [6]. While considering the only one factor such as the production site, there was a significant difference (p<0.05) between the values of ash recorded in cowpea seeds analysed. It was the case of the protein content. The lowest values of ash (3.55±0.53%) and protein (20.88±0.07) were recorded in *Tonkpi* site while the highest ones, reached respectively 4.05±0.13% and 22.08±1.35% in *Marahoue* site. Carbohydrates variation is also revealed to be statistically significant (p<0.05) with highest value recorded in *Tonkpi* site (64.42±0.79%). In addition, the comparison between the physicochemical parameters of cowpea seeds depending on the coat colour revealed that several characteristics permit distinguishing these samples. Thus, the red cowpea recorded the highest values of energy and carbohydrates while the white seeds recorded the highest protein content and the lowest moisture. Variations in physicochemical characteristics of cowpea, depending on seed coat colour have been mentioned (Henshaw, 2008; Hamid *et al*., 2015) [6, 16].

The significant (p<0.05) effects of seed coat colour, production site and their interaction on physicochemical properties of cowpea seeds are responsible of differences observed between samples analysed. These differences between cowpea samples were observed or presented in the PCA plot. The analysis showed that cowpea samples from *Marahoue* site which seed coat is white recorded the highest protein content. The high protein content shows that these samples are a good raw material for the preparation of protein concentrate and for fighting against protein-deficiency diseases. Those from *Tonpki* site have relatively high moisture content which is not a serious issue as it is in the recommended range (less than 13%) for seed storage.

5. Conclusion

The findings of this study show that the cowpea seeds are rich in proteins and carbohydrates. They are energizing foods. It has clearly showed that differences exist between samples analysed. The physicochemical characteristics of cowpea seeds were affected significantly by both coat colour and production site. The effect of their interaction is also shown to be significant. The red cowpea recorded the highest values of energy and carbohydrates while the white cowpea seeds recorded the highest protein content and the lowest moisture. Cowpea samples from *Marahoue* site which seed coat is white recorded the highest protein content while the red ones collected in *Tonkpi* site recorded highest value of carbohydrates. The significant (P<0.05) effects of seed coat colour, production site and their interaction on physicochemical properties of cowpea seeds are responsible of differences observed between samples analysed.

6. Nomenclature

BM1: 1st sample of cowpea from *Marahoue* site with white seed coat
BM2: 2nd sample of cowpea from *Marahoue* site with white seed coat
BT1: 1st sample of cowpea from *Tonkpi* site with white seed coat
BT2: 2nd sample of cowpea from *Tonkpi* site with white seed coat
BP1: 1st sample of cowpea from *Poro* site with white seed coat
BP2: 2nd sample of cowpea from *Poro* site with white seed coat
RM1: 1st sample of cowpea from *Marahoue* site with red seed coat
RT1: 1st sample of cowpea from *Tonkpi* site with red seed coat
RP1: 1st sample of cowpea from *Poro* site with red seed coat

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