Use of Triple Bagging Systems and Lippia Multilora Leaves for the Protein-Energy Quality Preservation of Cowpea Seeds (*Vigna unguiculata L. Walp*).

FOFANA Ibrahim[1], CHATIGRE K. Olivier[2], KONAN K. Constant[3], BIEGO Godi Henri Marius[4]

[1-4]Laboratory of Biochemistry and Food Science, Training and Research Unit of Biosciences, Felix HOUPHOUET-BOIGNY University of Abidjan, 22 BP 582 Abidjan 22, Cote d'Ivoire

[4]Department of Public Health, Hydrology and Toxicology, Training and Research Unit of Pharmacological and Biological Sciences, Felix HOUPHOUET-BOIGNY University, BP 34 Abidjan, Cote d'Ivoire

Abstract— *Cowpea is a food legume highly appreciated in the traditional diet of the populations in Ivory Coast. It is confronted with storage and / or preservation problems which prevent his production in large quantity. This experiment carried out in Ivory Coast, made it possible to evaluate the effectiveness of the triple bagging systems combined or not with the use of Lippia multiflora leaves on maintaining the cowpea seeds protein-energy quality during preservation. Thus, a 6x6 factorial design was considered to evaluate the cowpea seeds protein-energy quality. The first factor consisted to six types of packaging namely: one control with polypropylene bag (TST), one triple bagging batch (composed of 2 internal layers in independent high density polyethylene 80 mm thick and a woven bag polypropylene) without biopesticide (H0), and four batches (H1, H2, H3 and H4) containing respectively (0.7%; 2.5; 4.3%; and 5%) biopesticide. And second factor storage time, it included six periods of observation (0; 1; 2; 4.5; 7 and 8 months). Results showed significant influence of the interaction between types of treatments and storage time upon protein-energy quality of cowpea. Indeed, the polypropylene control was destocked at 4.5 months and very significant changes were recorded in the protein-energy quality of the cowpea seeds. Also, in the triple bagging systems without biopesticide significant changes were observed to seven months of storage in the contents of moisture (10.03% to 12.55%); ash (2.73% to 2.40%); fiber (5.15% to 4.37%); lipids (1.86% to 1.40%); proteins (22.75% to 15.21%); starch (53.80% to 42.27%); total carbohydrates (62.62% to 68.44%); total sugars (15.89% to 8.15%); reducing sugars (3.12% to 2.20%) and energy value (358% to 347.20%). However, cowpea seeds stored in triple bagging systems with biopesticide retain protein energy characteristic better for eight months period. The average values of analysis tests remained around: 12.06% humidity; 2.60% ash; 4.98% fiber; 18.50% proteins; 1.73% lipids; 52.13% starch; 65.13% total carbohydrates; 14.10% total sugars; 3.00% reducing sugars and finally 350.05% energy value. The results of the multivariate analysis indicate that the 0.7% biopesticide concentration is effective and maintains the protein-energy quality of the cowpea seeds for up to 8 months.*

Keywords— Cowpea, preservation, triples bagging, biopesticide, protein energy characteristics.

I. INTRODUCTION

For more than 3500 years, cowpea (*Vigna unguiculata* L. Walp) has been one of most important legumes in nutrition of the peoples of African, Asian and Mediterranean continents (Bliss, 1972). Today, with an annual world production of 6.4 million tons (Nteranya and David, 2015), this food legume, consumed in various forms (donuts, boiled, mash, dough and sauce) is very popular throughout Africa because seeds are a valuable source of protein, less expensive for most populations. Indeed, cowpea seeds contain essential amino acids (Smart, 1964; Hignard, 1998; Archana and Jawali, 2007). Moreover, they provide a quantity of 3400 calories (Mukendi *et al*., 2014). Cowpea seeds are also an important source of carbohydrates, in particular dietary fiber (Bliss, 1972). Thus, the relatively balanced nutritional characteristics of cowpea seeds make it a very useful supplement in the diet. They are able to solve the problems of malnutrition and more specifically protein-energy deficiencies in sub-Saharan Africa. Furthermore, they are rich in micronutrients (essential minerals and vitamins) essential for the proper functioning of the body.

However, despite its importance, cowpea is faced with storage and / or conservation problems mainly due to attacks caused by pests such as bruchids (Agyen-Sampong, 1978; Doumma *et al*., 2011). This situation is supported by the lack of mastery of good post-harvest practices. In addition, inadequate storage makes the seeds vulnerable to microorganisms (fungi and storage bacteria) which qualitatively and quantitatively deteriorate stored grains (Bhushan *et al*., 2016; Konan, 2017). These microorganisms negatively affect protein energy quality (protein levels, starch, fatty acids, reducing sugars, non-reducing sugars and energy value) of the stored grains.

In order to cope with these stock destroyers, producers often resort to synthetic pesticides whose bad practices (misuse, lack of precaution in their handling and failure to meet the waiting periods for deficiency) can lead to the resistance of pests and diseases to environmental and health problems (Kétoh, 1998).

Given the extent of the damage caused by the use of these chemicals, the use of biopesticide as an alternative has been encouraged in recent decades (Bambara *et al*., 2008; Gueye *et al*., 2011; Kayombo *et al*., 2014).

Indeed, use of plants and their derivatives to treat and protect food is a very old practice in rural areas. It is an effective means of control, guarantees biodiversity and is less expensive (Regnault-Roger, 2002; Ketoh *et al*., 2005; Isman, 2006; Gueye *et al*., 2011). Among the aromatic plants used, is *Lippia multiflora*. It is a local plant and accessible in every region of Ivory Coast whose insecticidal and / or insect repellent properties have been revealed by recent cowpea preservation works (Illiassa, 2004; Tatsadjieu *et al*., 2009; Ilboudo *et al*., 2010; Konan, 2017).

Triple bagging systems are also frequently used in the preservation of cereals and legumes, including cowpea. They consist of a double layer of high density independent polyethylene placed inside a polypropylene woven bag. These systems have shown their effectiveness to extend the shelf life of cowpea seeds (Moussa *et al*., 2009; Baoua *et al*., 2012; De Groote *et al*., 2013; Vales *et al*., 2014; Mutambuki *et al*., 2015; Mutungi *et al*., 2016).

However, there are no recorded scientific data on evolution of protein-energy characteristics of cowpea seeds preserved in triple bagging systems to our knowledge in Ivory Coast. Thus, the purpose of the present working is to evaluate effects of triple bagging systems combined or not with use of *Lippia multiflora* leaves (biopesticide) on evolution of the biochemical characteristics (protein-energy quality) of cowpea seeds during the preservation.

II. MATERIAL AND METHOD

2.1 Experimental site

The experiment was carried out at the Laboratory of Biochemistry and Food Sciences (LaBSA) of the UFR Biosciences at the University Felix HOUPHOUET-BOIGNY. The different bags were stored in a laboratory storage room at 28.0 ± 0.2ºC of temperature and 75.0 ± 1.0% relative humidity. Wooden pallets have been placed on the floor as a support for the various types of packaging bags.

2.2 Biological material

Cowpea seeds used belong to the local variety "Vya". They were collected from producers in the Loh-Djiboua region (5° 50′ North 5° 22′ West) from April to May 2015 just after harvest. After hulling, the seeds have not undergone any treatment were sent to the laboratory for their packaging.

The leaves of *Lippia multiflora* were collected in Gbeke region in May 2015. They were dried out of the sun and then chopped in fine particles.

2.3 Storage equipment

Storage bags used were constituted polypropylene bags and triple bagging systems. The triple bagging systems obtained from the suppliers were composed of two internal layers of polyethylene liners (composed of 80 mm high density) and a third layer made from woven polypropylene. The two layers polyethylenes, one adapted inside the other, were enclosed in the polypropylene woven bag.

2.4 Protocol of cowpea seeds preserving

The experimentation was carried out from June 2015 to February 2016. It was implemented using the methodology of preservation by bagging cowpea seeds suggested by Konan *et al*., (2016) modified.

These authors using a central composite design with five levels represented by two factors (shelf life 1 to 8 months and proportion of biopesticide 0 to 5%) followed the evolution of merchantability and health quality during the storage in triple bagging systems. Thus, in our study one control batch and five experimental batches were constituted. The control group consisted of cowpea seeds put in polypropylene bags (TST). For the five experimental batches, they included one lot containing cowpea seeds in triple bagging systems without biopesticide (H0) and four batches of cowpea seeds packed in triple bagging systems with different concentrations (H1: 0.7%); H2: 2.5%; H3: 4.3% and H4: 5%) chopped dried leaves of *Lippia multiflora*. The filling of the bags was made by alternating cowpea seeds and leaves as stratum. The mass of each bag was 50 kg.

2.5 Sampling

Sampling for analysis was carried out at different storage periods (Konan *et al*., 2016). The first analysis was done just before the conditioning for conservation (0 months). The aim was to determine base values (references) and then compare them to values obtained during preservation. Then cowpea samples (2.5 kg) were taken in triplicate at 1; 2; 4.5; 7 and 8 months. Bag sampling was done randomly. The samples were then milled in a hammer mill in the laboratory to obtain a fine grind to determine the biochemical parameters (protein-energy quality).

2.6 Biochemical analysis of samples

Proximate analyses were carried out using standard methods AOAC (2000) to determine biochemical changes. Thus, moisture, ash, fiber, lipid, protein, total sugars, reducing sugars, total carbohydrates, starch and energy values were determined. All analyzes were performed in triplicate.

Thus, cowpea moisture was deduced after drying 10 g of the samples in an oven (MEMMERT, Germany) at 105°C until a constant weight was obtained.

The ash content resulted from incineration of 5 g of the cowpea dried sample at 550 ° C in an oven (PYROLABO, France) for 12 h until a light gray ash occurred.

For crude fibers, 2 g of crushed cowpea samples were taken. Then, extraction mixture was prepared using 0.25 M sulfuric acid and 0.31 M sodium hydroxide with intermittent boiling. After suction filtration, the insoluble residue was washed with hot water, dried with an oven (MEMMERT, Germany) at 100°C for 2 h then incinerated. The final residue allowed estimation of the crude fibers content.

The proteins contents were determined with use of the Kjeldhal method.

The lipids contents resulted from a solvent (hexane) extraction using a Soxhlet device.

Starches contents were determined using iodine method of Jarvis and Walker (1993).

Total soluble sugars amounts were determined by the method of Dubois *et al*. (1956) with phenol and sulfuric acid, then reducing sugars were measured out according to the method of Bernfeld *et al*. (1955) basing on the 3, 5-dinitrosallicylic acid reagent. Prior to their quantification; sugars were extracting with ethanol, zinc acetate and oxalic acid (Agbo *et al.*, 1985). Total carbohydrate and energy value were estimated using the following formulas (FAO, 2002):

Carbohydrates (%) = 100 - (% moisture + % proteins + % lipids + % ash).

Energy (%) = (% proteins X 4) + (% carbohydrates X 4) + (% lipids X 9).

The results of proteins, lipids, ash, fiber, starch, total carbohydrates content, total soluble and reducing sugars were expressed on the dry weight basis.

2.7 Statistical analysis

The statistical analyzes of data were carried out thanks to software SPSS (version 22.0) and STATISTICA (version 7.1). All assays for biochemical characteristics were performed in triplicate and the results are expressed as mean ± standard deviation. A repeated measure ANOVA (ANOVA mixed) was first performed on all the results during the first four and a half months of conservation. It consisted in Analysis of Variance according to two factors: duration and type of treatments and then completed by a one-way Analysis of Variance (ANOVA 1) for the rest of conservation period (7 and 8 months). The purpose of these tests was to determine the existence of significant statistical differences between the calculated mean values. The significant statistical differences were highlighted by the Tukey test at 5% significance level. Finally Correlations between parameters were also assessed according to the Pearson index. Then, Multivariate Statistical Analysis (MSA) namely Principal Components Analysis (PCA) and Ascending Hierarchical Classification analysis (AHC) were performed.

III. RESULTS

3.1 Evolution of the nutritive parameters (protein energy quality) of cowpea seeds according to treatments during preservation

The statistical traits used to evaluate all biochemical parameters during storage are indicated in Tables (I, II and III). These tests reveal significantly changes (P <0.05) in the content of these parameters assessed according to the duration and type of treatments; whether or not the cowpea seeds were stored using the triple bagging method and whether they were treated or not treated with the biopesticide.

3.1.1 Moisture content

Tables IV and V show the moisture of cowpea seeds stored according to treatments. With mean of 10.03 % at the beginning (0 month), the moisture contents increases significantly (P <0.001) during the storage period. The highest moisture values were recorded after 4.5 months of storage in the control polypropylene bag with a mean of 14.67%. In the triple bagging system without biopesticide, from the 7th month of storage, this rate increased rapidly (12.55%) to 8 months of storage, the value of 14.10% (Table V). While in triple bagging systems with different proportions of biopesticide, the moisture content of cowpea seeds remained around 12.06% during the 8 months of storage (Table V). Furthermore, the interaction between the type of treatments and storage periods has a significant effect (P <0.001) upon this parameter (Table II).

3.1.2 Ash, fibers, protein and lipid contents

In triple bagging systems with different proportions of biopesticide, ash content remained constant during the 8 months of storage with a mean of 2.60% (Table V). The values of the ash content in the triple bagging system without biopesticide (H0) decrease significantly (P <0.001) after 2 months of storage (Table IV) to reach a value of 2.40% in month 7 (Table V). As for the control polypropylene bag (TST) the change is significant during the 4.5 months of storage thus increasing from 2.73% to 1.80%.

On the other hand, the lipids contents do not change significantly with the storage periods for triple bagging systems containing biopesticide (Table IV and V). Similar comments have been made for fiber and protein contents in these storage systems (Table IV and V). It is at the end of the 8th month of storage, the decrease for protein content becomes appreciable in the different triple bagging systems containing biopesticide, whereas the change for the fiber contents become significant (P <0.001) in the triple bagging with 0.7% biopesticide. However, in both types of bags without biopesticide, these macronutrients (fiber, lipids and proteins) change significantly (P <0.001) with the duration, type of treatments and the interaction between these two variables with a strong emphasis for the control (TST). Indeed, with a mean value of 22.75% at the beginning of storage (0 month), the protein content of cowpea seeds drops to 11.23% in the polypropylene bag (control) after 4.5 months (destocking period) and 15.21% in the triple bagging system without biopesticide after 7 months of storage. Similarly, in the control bag (TST) and the triple bagging without biopesticide (H0) the fiber and lipid contents decreased significantly (P <0.001) from 5.15% of fibers at the beginning to 4.07% at the end of 4.5 months of storage for control TST and to 4.37% and 3.73% respectively after 7 and 8 months of storage for H0. Regarding lipids, the rates of 1.86% at the beginning drop to 1.07% at 4.5 months for the control polypropylene and to 1.40% and 1.18% at 7 and 8 months for H0 bags.

3.1.3 Starch and total carbohydrates contents

The starches contents are significantly influenced (P <0.001) by the duration and type of treatments, also by the interaction between the two variables (Tables I and II).

A gradual decrease is observed with storage periods for two types of bags without biopesticide (TST and H0). The starches contents of cowpea seeds at the earlier storage 53.80% drop to 40.43% in the polypropylene control (TST) during the 4.5 months of storage (Table VI). In the triple bagging system without biopesticide (H0) this rate drops to 42.27% after 7 months of storage and 41.65% after 8 months of storage. For the other types of treatment (triple bagging with different proportions of biopesticide), no significant difference was revealed during the entire storage period (Table VI and VII).

For total carbohydrates, a significant change (P <0.001) in all samples was observed with an increasing trend. This rise is more pronounced in the samples (TST and H0). These rates vary respectively from 62.62% to 71.23% (TST) after 4.5 months of storage (Table VI) and from 62.62% to 68.44% (H0) after 7 months of storage. In the various triple bagging systems with biopesticide, the mean fluctuate between 64.61% and 65.13% after 8 months of storage (Table VII).

3.1.4 Total and reducing sugars

The post harvest cowpea storage revealed a significant decrease (P <0.001) in the total sugars contents during storage. This decrease is more marked in the control group and the triple bagging system without biopesticide (Tables VI and VII). During the 4.5 months of the polypropylene control storage, a drop from 15.89% at the beginning of storage to 6.99% in the month of destocking (month 4.5) was recorded. Similarly, in the triple bagging system without biopesticide, a drop of 15.89% to 8.15% and 6.70% was observed respectively after 7 and 8 months of storage. Total sugars contents for triple bagging with 0.7% biopesticide (H1) decreased significantly from 15.89% (month 0) to 11.90% after 8 months of storage. For cowpea seeds treated with 2.5%; 4.3% and 5% of biopesticide no significant change was observed after 8 months of storage (Tables VII). Regarding reducing sugars, there is a significant difference (P <0.001) between the beginning and end of storage for the TST control and the H0 bag. The means change respectively from 3.12% to 1.51% after 4.5 months of storage and from 3.12% to 2.08% at 8 months of storage. For triple bagging systems with biopesticide there was no significant change between the types of treatments after 8 months of storage (Table VII).

3.1.5 Energy values

The energy values estimated at 358.25 kcal / 100g before storage. dropped significantly (P <0.001) after 4.5 months of storage (353.91 kcal / 100g) both in triple bagging systems with biopesticide and in the triple bagging system without biopesticide (Tables VI). However, after 8 months of storage this value reaches 340.70 kcal / 100g in the bag (H0) and remains around 350.05 kcal / 100g in the systems (H1, H2, H3 and H4) (Table VII).

For the TST control the significant decrease (P <0.001) was observed over the entire storage period (4.5 months) and the values at the end of storage were 339.47 kcal / 100g.

3.2 Correlations between nutritive parameters

The Pearson index (r) indicate positive and negative significant correlations between the 10 parameters assessed for cowpea samples untreated and from different treatments (Table VIII). Thus, ash, fibers, proteins, lipids, total sugars, reducing sugars, starch and energy value are closely correlated during storage of cowpea, with r varying from 0.80 to 0.98. Indeed, the contents of proteins and reducing sugars change tightly (r = 0.90). The starch contents are directly correlated with the fiber contents (r = 0.92). Positive significant correlations are observed between lipid contents and reducing sugars (r = 0.96). The ash content during storage is proportional to that of the reducing sugars (r = 0.96). The energy value is strongly influenced by protein and lipid contents. Conversely, changes in total carbohydrate contents are negatively correlated with protein, starch, fiber, lipid, reducing and total sugars, ash and energy content. In addition, the analysis also showed that an increase in the moisture content strongly coincides with a decrease of the 8 other biochemical parameters studied.

3.3 Variability between types of treatments and nutritive parameters during storage

Principal Component Analysis (PCA) was achieved with the main factors F1 and F2 (Table IX) delivering eigenvalue equal or superior to 1, according to statistical standard of Kaïser. Then, gatherings highlighted from the PCA were clarified by Ascending Hierarchical Classification (AHC) performed with the Unweighted Pair Group Method with Arithmetic means (UPGMA).

3.4 Multivariate analysis

Principal component analysis (PCA) correlated whole characters studied with two factors “Fig. 1.a”. However, only the first factor (F1) with an eigenvalue greater than 1 and cumulating 90.23% of the total variability of all parameters was considered for the PCA data interpretation. Thus the F1 factor of eigenvalue 9.02 is significantly positive correlated with moisture and total carbohydrate contents, but negatively with the protein, starch, fiber, ash, lipid, total sugars and reducing sugars, and the energy value. However, the factor F2 of eigenvalue 0.49 and of total variability 4.90 is associated with F1 for the PCA representation. The projection of the samples studied highlighted 3 groups of individuals “Fig. 1.b”. Indeed the samples from the first group have high moisture and total carbohydrates contents. Those in the second group recorded more significant levels of proteins, lipids, ashes, fibers, total and reducing sugars, starch and energy value. On the other hand, the samples of the third group are not specifically distinct from those of second group. However the Ascending hierarchical classification (AHC) shows a large class comprising individuals from both the second and third PCA groups “Fig. 2”. This shows that all individuals of the samples preserved with biopesticide respectively at 7 and 8 months (C4 to F5) are close to the second group, which are in fact the initial sample, just after harvest (EI), samples from 1 month of preservation (A1 to F1), also with the exception of the polypropylene control, those with 2 months of preservation (B2 to F2) and finally individuals from samples preserved with biopesticide at 4.5 months (C3 at F3).

When the individuals in the first group, they consist of control samples polypropylene at 2 months (A2), polypropylene and triple bagging without biopesticide at 4.5 months (A3-B3) and triple bagging without biopesticide at 7 and 8 months (B4 and B5).

TABLE 1
STATISTICAL DATA (MIXED ANOVA) OF PROTEIN-ENERGY CHARACTERISTICS OF COWPEA SEEDS UNDER TREATMENT DURING PRESERVATION

SOV	Stat. Para		PRC	LPC	STC	FBC	TSC	RSC	ASC	TCC	EC	MC
Durations	**Sphericity hypothesis**	**df**	3	3	3	3	3	3	3	3	3	3
		SS	63.434	0.550	165.801	0.602	68.868	1.593	0.580	59.380	522.298	30.417
		MS	21.145	0.183	55.267	0.201	22.956	0.531	0.193	19.793	174.099	10.139
		F	41.671	50.689	86.169	46.915	234.226	97.788	44.271	55.948	225.741	812.877
		P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Greenhouse-Geisser	**df**	2.525	1.806	2.807	2.490	2.371	2.180	2.200	2.743	1.939	1.433
		SS	63.434	0.550	165.801	0.602	68.868	1.593	0.580	59.380	522.298	30.417
		MS	25.120	0.305	59.066	0.242	29.047	0.731	0.264	21.646	269.386	21.220
		F	41.671	50.689	86.169	46.915	234.226	97.788	44.271	55.948	225.741	812.877
		P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Error	**Sphericity hypothesis**	**df**	36	36	36	36	36	36	36	36	36	36
		SS	18.267	0.130	23.090	0.154	3.528	0.195	0.157	12.736	27.764	0.449
		MS	0.507	0.004	0.641	0.004	0.098	0.005	0.004	0.354	0.771	0.012
	Greenhouse-Geisser	**df**	30.302	21.667	33.684	29.874	28.451	26.158	26.403	32.918	23.266	17.202
		SS	18.267	0.130	23.090	0.154	3.528	0.195	0.157	12.736	27.764	0.449
		MS	0.603	0.006	0.685	0.005	0.124	0.007	0.006	0.387	1.193	0.026

***SOV**: source of variation ; **Stat Para**: statistical parameters ; **df**: degree of freedom ; **SS**: sum of squares ; **MS**: mean squares ; **F**: value of the statistical test ; **P**: probability value of the statistical test ; **PRC**: protein contents ; **LPC**: lipid content ; **STC**: starch content ; **ASC**: ash content ; **FBC**: fiber content ; **TSC**: total soluble sugar content ; **RSC**: reducing sugar content ; **TCC**: total carbohydrate content ; **EC**: energy content ; **MC**: moisture content.*

TABLE 2
STATISTICAL DATA (MIXED ANOVA) OF PROTEIN-ENERGY CHARACTERISTICS OF COWPEA SEEDS UNDER TREATMENT DURING PRESERVATION

SOV	Stat. Para		PRC	LPC	STC	FBC	TSC	RSC	ASC	TCC	EC	MC
Treatments		df	5	5	5	5	5	5	5	5	5	5
		SS	219.834	1.249	320.503	1.880	143.376	4.911	1.663	130.568	376.229	22.597
		MS	43.967	0.250	64.101	0.376	28.675	0.983	0.333	26.114	75.246	4.519
		F	134.958	96.510	86.919	77.175	140.116	133.687	86.558	96.299	75.302	292.153
		P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Error		df	12	12	12	12	12	12	12	12	12	12
		SS	3.909	0.031	8.850	0.058	2.456	0.088	0.046	3.254	11.991	0.186
		MS	0.326	0.003	0.737	0.005	0.205	0.007	0.004	0.271	0.999	0.015
Durations x treatments	Sphericity hypothesis	df	15	15	15	15	15	15	15	15	15	15
		SS	230.064	0.640	291.212	1.587	127.105	4.127	1.115	119.405	338.509	22.474
		MS	15.338	0.043	19.414	0.106	8.474	0.275	0.074	7.960	22.567	1.498
		F	30.227	11.795	30.269	24.724	86.459	50.677	17.028	22.501	29.261	120.121
		P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Greenhouse-Geisser	df	12.626	9.028	14.035	12.448	11.855	10.899	11.001	13.716	9.694	7.167
		SS	230.064	0.640	291.212	1.587	127.105	4.127	1.115	119.405	338.509	22.474
		MS	18.221	0.071	20.749	0.128	10.722	0.379	0.101	8.706	34.919	3.136
		F	30.227	11.795	30.269	24.724	86.459	50.677	17.028	22.501	29.261	120.121
		P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

***SOV**: source of variation ; **Stat Para**: statistical parameters ; **df**: degree of freedom ; **SS**: sum of squares ; **MS**: mean squares ; **F**: value of the statistical test ; **P**: probability value of the statistical test ; **PRC**: protein contents ; **LPC**: lipid content ; **STC**: starch content ; **ASC**: ash content ; **FBC**: fiber content ; **TSC**: total soluble sugar content ; **RSC**: reducing sugar content ; **TCC**: total carbohydrate content ; **EC**: energy content ; **MC**: moisture content.*

TABLE 3
STATISTICAL DATA (ANOVA 1) OF PROTEIN-ENERGY CHARACTERISTICS OF COWPEA SEEDS UNDER TREATMENT DURING PRESERVATION

Effect	Stat para	PRC	LPC	STC	FBC	TSC	RSC	ASC	TCC	EC	MC
Treatments	df	4	4	4	4	4	4	4	4	4	4
	SS	63.081	0.744	297.700	4.157	151.065	2.161	0.389	39.340	224.000	10.592
	F	8.940	22.242	78.500	109.560	120.769	39.048	16.060	5.630	149.000	219.60
	P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.05**	**<0.001**	**<0.001**
Error	df	10	10	10	10	10	10	10	10	10	10
	SS	17.641	0.084	9.480	0.095	3.127	0.138	0.060	17.480	4.000	0.121
Total	df	14	14	14	14	14	14	14	14	14	14
	SS	80.722	0.828	307.18	4.252	154.192	2.299	0.449	56.820	228.000	10.713

***Stat Para**: statistical parameters ; **PRC**: protein contents ; **LPC**: lipid content ; **STC**: starch content ; **ASC**: ash content ; **FBC**: fiber content ; **TSC**: total soluble sugar content ; **RSC**: reducing sugar content ; **TCC**: total carbohydrate content ; **EC**: energy content ; **MC**: moisture content.*

TABLE 4
EVOLUTION IN PROTEIN ENERGY CHARACTERISTIC (MOISTURE, ASH, LIPID, PROTEIN AND FIBER) ACCORDING TO TREATMENT AT 4.5 MONTH OF STORAGE (ON DRY WEIGHT BASIS)

Parameters	Storage time (month)	TST	H0	H1	H2	H3	H4
Moisture (%)	0	10.03±0.21^{aA}	10.03±0.21^{aA}	10.03±0.21^{aA}	10.03±0.21^{aA}	10.03±0.21^{aA}	10.03±0.21^{aA}
	1	10.24±0.06^{aA}	10.09±0.02^{aA}	10.07±0.03^{aA}	10.03±0.06^{aA}	10.03±0.02^{aA}	10.03±0.06^{aA}
	2	12.30±0.10^{bB}	10.22±0.02^{aA}	10.10±0.05^{aA}	10.08±0.02^{aA}	10.08±0.02^{aA}	10.07±0.04^{aA}
	4.5	14.67±0.15^{cB}	11.17±0.06^{bA}	11.05±0.06^{bA}	11.01±0.03^{bA}	10.99±0.01^{bA}	10.99±0.01^{bA}
Ash (%)	0	2.73±0.06^{aA}	2.73±0.06^{aA}	2.73±0.06^{aA}	2.73±0.06^{aA}	2.73±0.06^{aA}	2.73±0.06^{aA}
	1	2.51±0.03^{aB}	2.57±0.03^{aB}	2.68±0.18^{aA}	2.70±0.12^{aA}	2.71±0.02^{aA}	2.71±0.03^{aA}
	2	2.10±0.10^{bC}	2.53±0.06^{bB}	2.66±0.05^{aA}	2.69±0.05^{aA}	2.70±0.02^{aA}	2.71±0.04^{aA}
	4.5	1.80±0.10^{cC}	2.48±0.03^{bB}	2.63±0.04^{aA}	2.67±0.02^{aA}	2.68±0.02^{aA}	2.69±0.01^{aA}
Lipid (%)	0	1.86±0.05^{aA}	1.86±0.05^{aA}	1.86±0.05^{aA}	1.86±0.05^{aA}	1.86±0.05^{aA}	1.86±0.05^{aA}
	1	1.53±0.06^{bB}	1.80±0.00^{aB}	1.82±0.03^{aAB}	1.83±0.06^{aA}	1.83±0.04^{aA}	1.84±0.03^{aA}
	2	1.38±0.03^{cC}	1.72±0.03^{bB}	1.80±0.02^{aAB}	1.80±0.01^{aA}	1.83±0.04^{aA}	1.83±0.05^{aA}
	4.5	1.07±0.12^{cD}	1.57±0.15^{bC}	1.73±0.03^{aB}	1.77±0.10^{aA}	1.79±0.01^{aA}	1.80±0.02^{aA}
Protein (%)	0	22.75±0.87^{aA}	22.75±0.87^{aA}	22.75±0.87^{aA}	22.75±0.87^{aA}	22.75±0.87^{aA}	22.75±0.87^{aA}
	1	22.50±0.46^{aA}	22.57±0.51^{aA}	22.60±0.53^{aA}	22.67±0.35^{aA}	22.67±0.74^{aA}	22.70±0.52^{aA}
	2	15.03±0.51^{bB}	22.21±0.25^{aA}	22.43±0.51^{aA}	22.58±0.51^{aA}	22.59±0.10^{aA}	22.66±0.49^{aA}
	4.5	11.23±0.90^{cB}	22.17±1.27^{aA}	22.37±0.34^{aA}	22.40±0.40^{aA}	22.43±0.67^{aA}	22.47±0.76^{aA}
Fiber (%)	0	5.15±0.04^{aA}	5.15±0.04^{aA}	5.15±0.04^{aA}	5.15±0.04^{aA}	5.15±0.04^{aA}	5.15±0.04^{aA}
	1	4.97±0.08^{aA}	5.13±0.06^{aA}	5.13±0.04^{aA}	5.13±0.06^{aA}	5.13±0.12^{aA}	5.15±0.05^{aA}
	2	4.60±0.10^{bB}	5.05±0.05^{aAB}	5.13±0.03^{aA}	5.13±0.03^{aA}	5.13±0.06^{aA}	5.15±0.05^{aA}
	4.5	4.07±0.15^{cC}	4.90±0.10^{bB}	5.10±0.02^{aA}	5.12±0.03^{aA}	5.13±0.06^{aA}	5.15±0.09^{aA}

*Means (±SD) with different upper-case/lower-case letters in the same line/column are different at 5% probability test. With **TST**: control polypropylene bag; **H0**: triple bagging without biopesticide; **H1**: triple bagging with 0.7% biopesticide (w / w); **H2**: triple bagging with 2.5% biopesticide (w / w); **H3**: triple bagging with 4.3% biopesticide (w / w); **H4**: triple bagging with 5% biopesticide (w / w).*

TABLE 5
EVOLUTION IN PROTEIN ENERGY CHARACTERISTIC (MOISTURE, ASH, LIPID, PROTEIN AND FIBER) ACCORDING TO TREATMENT AFTER 7 AND 8 MONTHS OF STORAGE (ON DRY WEIGHT BASIS)

Parameters	Storage time (month)	Treatments				
		H0	H1	H2	H3	H4
Moisture (%)	7	12.55±0.11[a]	11.72±0.03[b]	11.63±0.01[b]	11.56±0.19[b]	11.54±0.04[b]
	8	14.10±0.11[a]	12.10±0.10[b]	12.06±0.12[b]	12.06±0.06[b]	11.83±0.14[b]
Ash (%)	7	2.40±0.01[b]	2.58±0.02[a]	2.60±0.05[a]	2.61±0.05[a]	2.65±0.05[a]
	8	2.20±0.02[b]	2. 54±0.15[a]	2.58±0.08[a]	2.60±0.02[a]	2.64±0.04[a]
Lipid (%)	7	1.40±0.10[b]	1.70±0.02[a]	1.76±0.04[a]	1.76±0.09[a]	1.78±0.11[a]
	8	1.18±0.03[b]	1.69±0.10[a]	1.73±0.15[a]	1.74±0.05[a]	1.77±0.06[a]
Protein (%)	7	15.21±1.32[b]	22.23±0.40[a]	22.25±0.25[a]	22.33±0.58[a]	22.40±0.38[a]
	8	13.63±1.89[b]	18.14±0.99[a]	18.50±1.30[a]	18.98±1.38[a]	19.12±0.82[a]
Fiber (%)	7	4.37±0.11[b]	5.07±0.01[a]	5.12±0.03[a]	5.13±0.03[a]	5.14±0.04[a]
	8	3.73±0.15[c]	4.60±0.10[b]	4.98±0.10[a]	5.11±0.04[a]	5.14±0.04[a]

Means (±SD) with different lower-case letters in the same line are different at 5% probability test. With ***H0****: triple bagging without biopesticide;* ***H1****: triple bagging with 0.7% biopesticide (w / w);* ***H2****: triple bagging with 2.5% biopesticide (w / w);* ***H3****: triple bagging with 4.3% biopesticide (w / w);* ***H4****: triple bagging with 5% biopesticide (w / w).*

TABLE 6
EVOLUTION IN PROTEIN ENERGY CHARACTERISTIC (STARCH, TOTAL CARBOHYDRATE, TOTAL SOLUBLE SUGAR, REDUCING SUGAR AND ENERGY VALUE) ACCORDING TO TREATMENT AT 4.5 MONTH OF STORAGE (ON DRY WEIGHT BASIS)

Parameters	Storage time (month)	TST	H0	H1	H2	H3	H4
Starch (%)	0	53.80±0.68^{aA}	53.80±0.68^{aA}	53.80±0.68^{aA}	53.80±0.68^{aA}	53.80±0.68^{aA}	53.80±0.68^{aA}
	1	51.25±0.22^{bB}	53.43±1.16^{aA}	53.63±0.64^{aA}	53.67±0.71^{aA}	53.77±0.51^{aA}	53.80±1.37^{aA}
	2	46.22±0.95^{bB}	53.07±0.95^{aA}	53.57±0.25^{aA}	53.63±0.71^{aA}	53.70±1.18^{aA}	53.70±0.70^{aA}
	4.5	40.43±0.68^{cC}	45.57±0.65^{bB}	52.70±0.75^{aA}	53.30±0.96^{aA}	53.40±1.22^{aA}	53.66±0.78^{aA}
Total carbohydrate (%)	0	62.62±0.73^{aA}	62.62±0.73^{aA}	62.62±0.73^{aA}	62.62±0.73^{aA}	62.62±0.73^{aA}	62.62±0.73^{aA}
	1	63.22±0.52^{aA}	62.97±0.52^{aA}	62.83±0.45^{aA}	62.77±0.14^{aA}	62.76±0.68^{aA}	62.72±0.45^{aA}
	2	69.19±0.29^{bB}	63.31±0.30^{aA}	63.00±0.56^{aA}	62.84±0.46^{aA}	62.80±0.04^{aA}	62.73±0.38^{aA}
	4.5	71.23±1.02^{cB}	64.61±0.45^{bA}	64.22±0.42^{abA}	63.15±0.32^{abA}	63.10±0.63^{abA}	63.05±0.74^{abA}
Total soluble sugar (%)	0	15.89±0.19^{aA}	15.89±0.19^{aA}	15.89±0.19^{aA}	15.89±0.19^{aA}	15.89±0.19^{aA}	15.89±0.19^{aA}
	1	14.77±0.21^{bB}	15.00±0.10^{abA}	15.63±0.46^{aA}	15.70±0.70^{aA}	15.83±0.15^{aA}	15.87±0.15^{aA}
	2	10.70±0.17^{cC}	13.63±0.56^{bB}	15.57±0.11^{aA}	15.70±0.26^{aA}	15.80±0.26^{aA}	15.87±0.29^{aA}
	4.5	6.99±0.18^{dC}	10.67±0.73^{cB}	15.50±0.51^{aA}	15.50±0.56^{aA}	15.53±0.50^{aA}	15.83±0.15^{aA}
Reducing sugar (%)	0	3.12±0.02^{aA}	3.12±0.02^{aA}	3.12±0.02^{aA}	3.12±0.02^{aA}	3.12±0.02^{aA}	3.12±0.02^{aA}
	1	2.93±0.15^{aB}	3.10±0.10^{aA}	3.11±0.10^{aA}	3.11±0.04^{aA}	3.11±0.10^{aA}	3.12±0.11^{aA}
	2	2.00±0.10^{bC}	2.99±0.01^{bB}	3.10±0.06^{aA}	3.10±0.10^{aA}	3.11±0.10^{aA}	3.11±0.12^{aA}
	4.5	1.51±0.04^{dC}	2.65±0.11^{cB}	3.06±0.05^{aA}	3.08±0.06^{aA}	3.10±0.00^{aA}	3.10±0.09^{aA}
Energy (kcal/100g)	0	358.25±1.07^{aA}	358.25±1.07^{aA}	358.25±1.07^{aA}	358.25±1.07^{aA}	358.25±1.07^{aA}	358.25±1.07^{aA}
	1	356.69±0.10^{aA}	357.87±0.39^{aA}	358.08±0.86^{aA}	358.19±0.40^{aA}	358.21±0.08^{aA}	358.25±0.44^{aA}
	2	349.30±0.66^{bB}	357.62±0.12^{aA}	357.91±0.16^{aA}	357.96±0.34^{aA}	358.00±0.07^{aA}	358.06±0.24^{aA}
	4.5	339.47±1.55^{cB}	353.25±2.81^{bA}	353.91±0.25^{bA}	354.02±0.63^{bA}	354.13±0.16^{bA}	354.26±0.71^{bA}

*Means (±SD) with different upper-case/lower-case letters in the same line/column are different at 5% probability test. With **TST**: control polypropylene bag; **H0**: triple bagging without biopesticide; **H1**: triple bagging with 0.7% biopesticide (w / w); **H2**: triple bagging with 2.5% biopesticide (w / w); **H3**: triple bagging with 4.3% biopesticide (w / w); **H4**: triple bagging with 5% biopesticide (w / w).*

TABLE 7
EVOLUTION IN PROTEIN ENERGY CHARACTERISTIC (STARCH, TOTAL CARBOHYDRATE, TOTAL SOLUBLE SUGAR, REDUCING SUGAR AND ENERGY VALUE) ACCORDING TO TREATMENT AFTER 7 AND 8 MONTHS OF STORAGE (ON DRY WEIGHT BASIS)

Parameters	Storage time (month)	Treatments				
		H0	H1	H2	H3	H4
Starch (%)	7	42.27±0.95[b]	51.47±1.18[a]	53.00±0.20[a]	53.30±1.04[a]	53.43±1.00[a]
	8	41.65±1.21[b]	51.18±1.69[a]	52.13±1.21[a]	53.13±0.42[a]	53.20±0.44[a]
Total carbohydrate (%)	7	68.44±1.12[a]	64.76±0.39[b]	63.76±0.23[b]	63.74±0.53[b]	63.63±0.31[b]
	8	68.89±1.79[a]	65.13±0.85[b]	65.13±1.37[b]	64.61±1.36[b]	64.64±1.04[b]
Total soluble sugar (%)	7	8.15±0.75[c]	13.29±0.47[b]	15.23±0.27[a]	15.30±0.10[a]	15.77±0.10[a]
	8	6.70±0.75[c]	11.90±0.10[b]	14.10±0.95[a]	15.07±0.21[a]	15.20±0.20[a]
Reducing sugar (%)	7	2.20±0.10[b]	3.03±0.06[a]	3.06±0.06[a]	3.08±0.06[a]	3.10±0.10[a]
	8	2.08±0.08[b]	2.90±0.10[a]	3.00±0.20[a]	3.07±0.06[a]	3.10±0.10[a]
Energy (kcal/100g)	7	347.20±0.09[b]	351.32±0.10[a]	351.86±0.11[a]	352.11±0.58[a]	352.12±0.45[a]
	8	340.70±0.36[b]	350.05±0.55[a]	350.05±0.97[a]	350.23±0.49[a]	351.00±0.52[a]

*Means (±SD) with different lower-case letters in the same line are different at 5% probability test. With **H0**: triple bagging without biopesticide; **H1**: triple bagging with 0.7% biopesticide (w / w); **H2**: triple bagging with 2.5% biopesticide (w / w); **H3**: triple bagging with 4.3% biopesticide (w / w); **H4**: triple bagging with 5% biopesticide (w / w).*

TABLE 8
MATRIX OF CORRELATIONS BETWEEN BIOCHEMICAL PARAMETERS OF COWPEA SEEDS DURING STORAGE

	PRC	LPC	STC	FBC	RSC	TSC	TCC	ASC	EC	MC
PRC	1.00									
LPC	0.88	1.00								
STC	0.85	0.95	1.00							
FBC	0.90	0.93	0.92	1.00						
RSC	0.90	0.96	0.95	0.90	1.00					
TSC	0.87	0.94	0.98	0.95	0.93	1.00				
TCC	-0.96	-0.93	-0.92	-0.89	-0.95	-0.92	1.00			
ASC	0.89	0.95	0.88	0.86	0.96	0.88	-0.93	1.00		
EC	0.89	0.84	0.81	0.83	0.80	0.83	-0.90	0.81	1.00	
MC	-0.89	-0.82	-0.79	-0.82	-0.79	-0.81	0.90	-0.81	-0.99	1.00

*The parameters values are significant at P=0.05; **PRC**: protein content; **LPC**: lipid content ; **STC**: starch content ; **ASC**: ash content ; **FBC**: fiber content ; **TSC**: total soluble sugar content ; **RSC**: reducing sugar content ; **TCC**: total carbohydrate content ; **EC**: energy content ; **MC**: moisture content.*

TABLE 9
EIGEN VALUES AND CORRELATION MATRICE FACTORS OF PRINCIPAL COMPONENTS ANALYSIS WITH BIOCHEMICAL PARAMETERS OF COWPEA STORED STUDIED

Factors	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Eigenvalues	9.02	0.49	0.21	0.12	0.08	0.04	0.02	0.01	0.00	0.00
Variances (%)	90.23	4.90	2.11	1.20	0.83	0.40	0.20	0.11	0.18	0.00
Cumulative variance (%)	90.23	95.14	97.25	98.45	99.28	99.68	99.86	99.97	100.00	100.00
PRC	**-0.95**	0.12	0.10	-0.25	0.04	0.02	0.05	-0.04	-0.00	-0.00
LPC	**-0.97**	-0.15	0.01	0.11	-0.10	0.10	0.07	0.01	-0.01	0.00
STC	**-0.95**	-0.21	-0.14	0.09	0.12	0.04	0.00	-0.03	0.03	0.00
FBC	**-0.95**	-0.10	-0.21	-0.15	-0.16	0.02	-0.05	0.02	0.01	0.00
RSC	**-0.96**	-0.20	0.13	0.01	0.05	0.05	-0.08	-0.02	-0.02	-0.00
TSC	**-0.96**	-0.16	-0.18	0.01	0.06	-0.13	0.03	0.01	-0.02	-0.00
TCC	**0.98**	-0.05	-0.12	0.04	-0.11	-0.01	0.00	-0.08	-0.01	-0.00
ASC	**-0.94**	-0.11	0.26	0.07	-0.11	-0.10	-0.00	-0.01	0.02	-0.00
EC	**-0.92**	0.39	-0.07	0.08	-0.01	0.01	-0.01	-0.01	-0.00	-0.01
MC	**0.91**	-0.41	0.03	-0.08	0.01	0.01	0.02	0.01	0.00	-0.01

*Values of significant correlations in bold at P=0.05; **PRC**: protein contents; **LPC**: lipid content; **STC**: starch content ; **ASC**: ash content ; **FBC**: fiber content ; **TSC**: total soluble sugar content ; **RSC**: reducing sugar content ; **TCC**: total carbohydrate content ; **EC**: energy content ; **MC**: moisture content.*

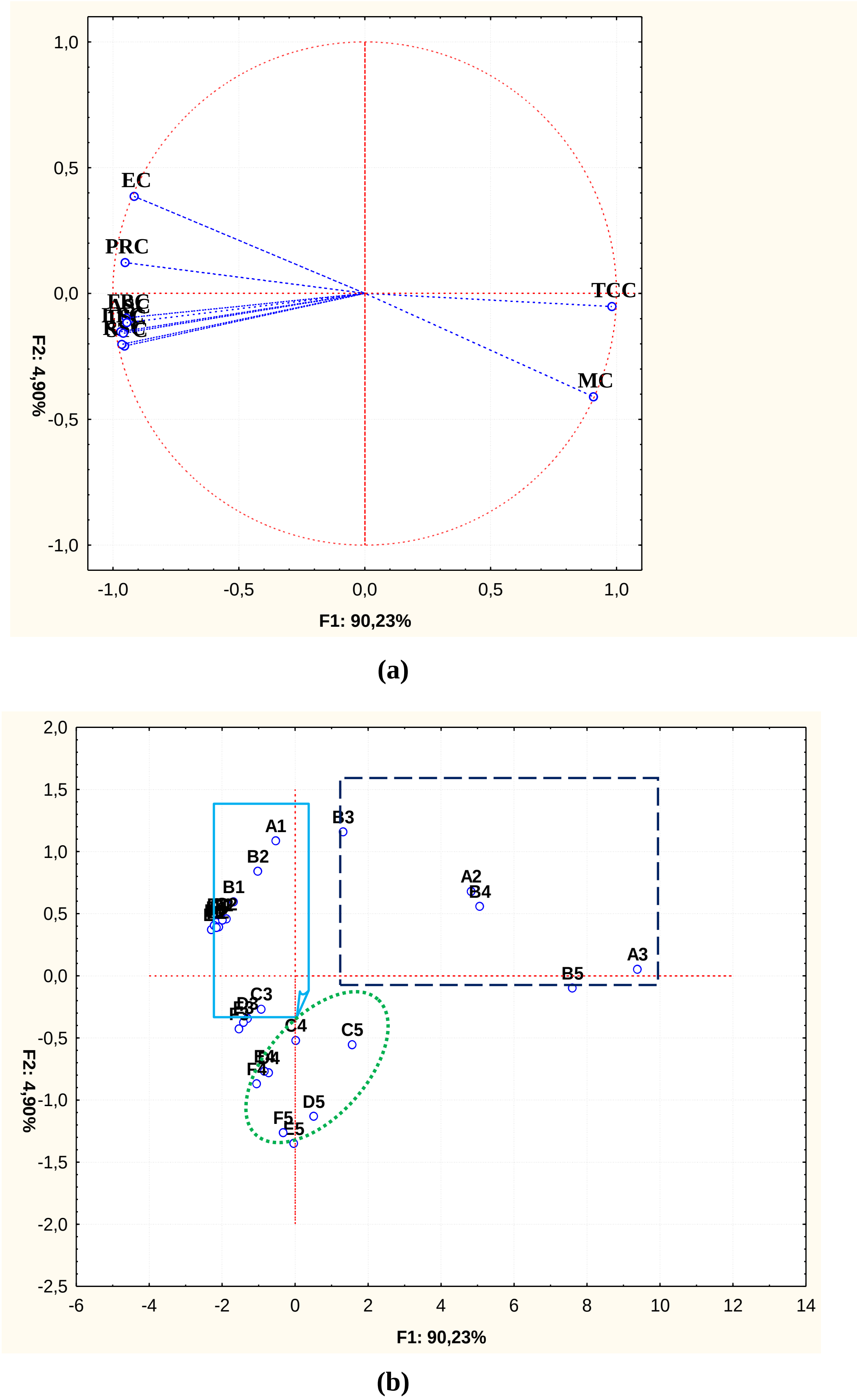

FIGURE 1: Correlation drawn between the F1-F2 principal component and the protein energy characteristics (a) and the types of individuals (b) deriving from the cowpea samples studied

PRC: protein contents; **LPC**: lipid content; **STC**: starch content; **ASC**: ash content; **FBC**: fiber content; **TSC**: total soluble sugar content; **RSC**: reducing sugar content; **TCC**: total carbohydrate content; **EC**: energy content; **MC**: moisture content.

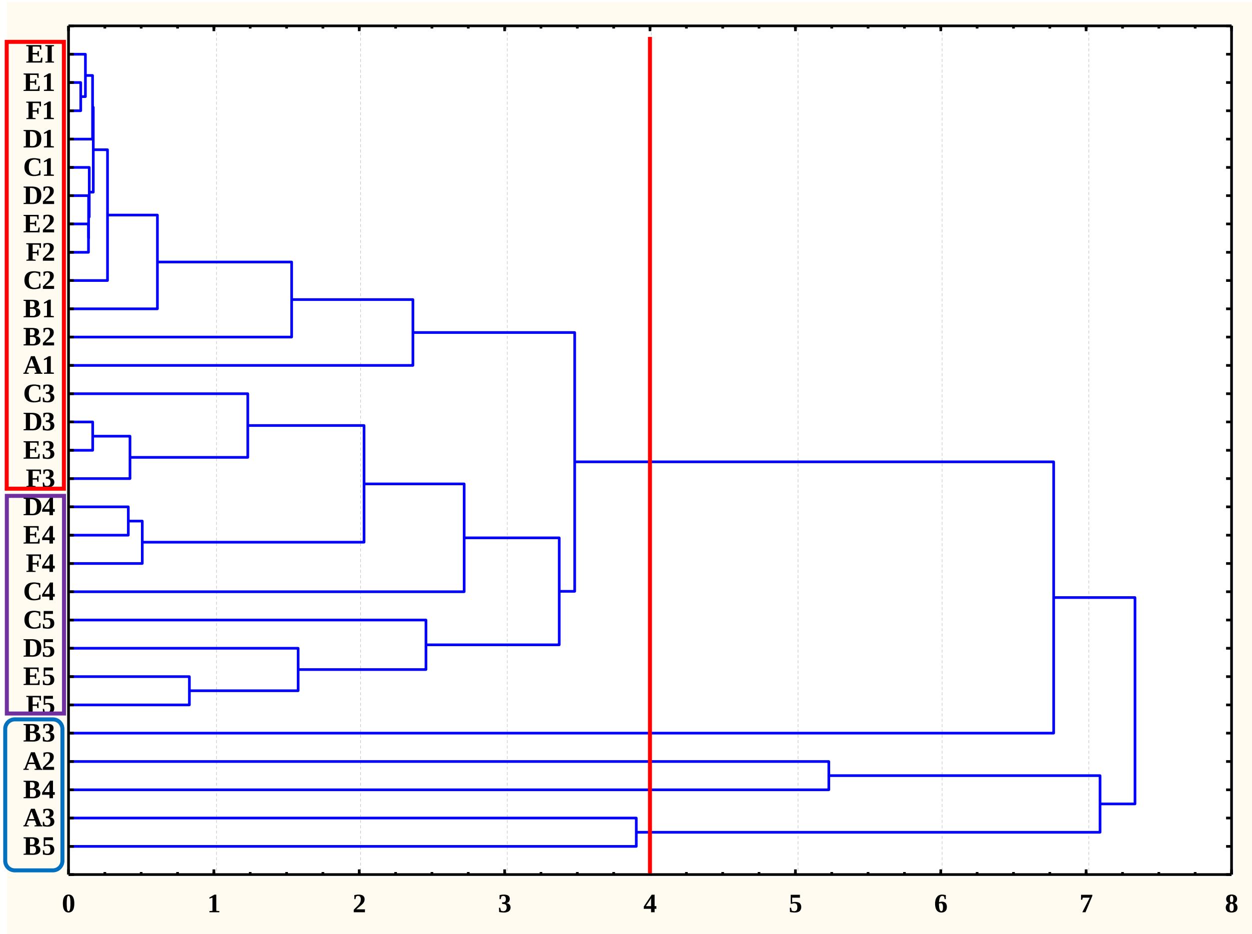

FIGURE 2: Ascending hierarchical notation (dendrogram) with the protein energy characteristics of cowpea preserved according different treatments

Ei: initial sample, **A1**: polypropylene bag at 1 month, **B1**: triple bagging without biopesticide at 1 month, **C1, D1, E1, F1**: triple bagging with 0.7%, 2.5%, 4.3% and 5% of biopesticide at 1 month **A2**: polypropylene bag at 2 months, **B2**: triple bagging without biopesticide at 2 months, **C2, D2, E2, F2**: triple bagging with respectively 0.7%, 2.5%, 4.3% and 5% biopesticide at 2 months of conservation. **A3**: polypropylene bag at 4.5 months, **B3**: triple bagging without biopesticide at 4.5 months, **C3, D3, E3, F3**: triple bagging with respectively 0.7%, 2.5%, 4.3% and 5% biopesticide at 4.5 months of storage. **B4**: triple bagging without biopesticide at 7 months, **C4, D4, E4, F4**: triple bagging with 0.7%, 2.5%, 4.3% and 5% biopesticide at 7 months of storage. **B5**: triple bagging without biopesticide at 8 months, **C5, D5, E5, F5**: triple bagging with 0.7%, 2.5%, 4.3% and 5% biopesticide at 7 months of storage.

IV. DISCUSSION

In order to maintain the protein-energy quality of cowpea seeds, the results obtained in this study show that the preservation of cowpea seeds in triple bagging systems with *Lippia multiflora* leaves is effective. The presence of *Lippia multiflora* leaves in these storage systems has allowed the moisture content of cowpea seeds to be maintained at recommended seed storage rates (8% to 12%) (Ahenkora *et al.*, 1998; Madamba, 2002). These leaves would act as films above the seeds thus protecting them against dehydration, the resumption and increase of moisture. This is supported by the results of Boeke *et al* (2004) and Konan *et al* (2016), who respectively showed that the dried leaves of *Momordica charantia* and *Lippia multiflora* are effective against the weight loss of stored cowpea.

Niamketchi *et al* (2016) have also shown that the combination of the leaves of two plants, *Lippia multiflora* and *Hiptis suaveolens* makes it possible to keep the moisture content of corn kernels at a minimum during storage. On the other hand, in the TST and H0 bags, a rise in the moisture content of the seeds could be due to an increase in the air relative humidity (Di Domenico *et al.*, 2015) in view of the partial impermeability of these storage systems (Lange and Wyser, 2003). This increase in seed moisture content would also be related to an increased population of fungi and insects and their metabolism during storage. Environmental moisture is produced by the respiration processes and could affect the moisture content of the stored grain. Previous research associates significant increases in seed moisture content during storage to the bioactivities from insects and fungi (Rubasingheye *et al*., 2007, Maalekuu and Kotey, 2014). The preservation technique using triple bagging combined with the different proportions (0.7 to 5%) of biopesticide (*Lippia multiflora*) is effective in comparison with the control (single polypropylene bag) and triple bagging without biopesticide. Significant increase in seed moisture content due to humidity, insects and fungi in polypropylene bags at 2 months and in triple bagging systems without biopesticide at 7 months compared to the slowed increase in triple bagging systems with biopesticide demonstrates the effectiveness of Lippia leaves. This finding was made by Niamketchi *et al*. (2016) and Konan *et al* (2016).

The significant differences found in the macronutrients determined respectively in the polypropylene bags, the triple bagging systems with or without biopesticide, also showed the degree of effectiveness of the triple bagging systems and biopesticide (*Lippia multiflora*) in the cowpea preservation. Indeed, the hierarchical ascending classification (HAC) has shown that the macronutrient contents of cowpea seeds stored for 8 months in triple bagging systems with at least 0.7% of biopesticide remain similar overall to those of cowpea after harvest.

Results obtained from the contents of ash, fiber, lipid and protein from the various experiments are similar to the investigations of Ojiako and Kayode (2014) on cowpea seeds stored under different conditions. These authors reported a decrease in the ash, fiber and protein contents, as well as the constant maintenance of the lipid content according to the plant used during storage. Similar changes were also mentioned by Mbah and Silas (2007), Maalekuu and Kotey (2014), Sule *et al* (2016) about the evaluation of cowpea seeds quality attributes in different types of storage. But according to them, the consumption of organic compounds through metabolism of grain and associated microorganisms could increase the ash content during storage. This fact is also contradictory with the results of Houinsou *et al* (2014) which showed that cowpea seeds after 3 months of storage do not record a significant change in their ash content.

The reduction in lipids contents would derive from the degradation occurring during storage and is related to biochemical processes such as respiration, oxidation and enzymatic activity (Paraginski *et al*., 2014). Decreased of lipid contents may also be due to insects that use it as a source of energy (Maalekuu and Kotey, 2014) and fungal attacks in seeds during storage (Chatta *et al*., 2015). These results corroborate those of Aremu *et al* (2015) who found a significant reduction in the percentage of lipids contents of cowpea seed stored for 16 weeks due to microbial food that takes place within the cowpea tissue at as the duration increases. However, Ojiako and Kayode (2014) showed that storage had no effect on the initial and final fat contents of cowpea seeds treated with natural (plant) and synthetic insecticides.

The loss of protein content would be related to changes in moisture content during storage considering the opposite correlation between both parameters. Changes in protein content may result from intrinsic chemical degradation of the seeds and / or their needs (Paraginski *et al*., 2014; Stefanello *et al*., 2015). The decline in the protein content of cowpea during storage could be related to the portion of the seeds consumed by associated insects and microorganisms, since in the polypropylene control bags after 4.5 months of storage, the insects have almost totally damaged the cowpea seeds and at the same time, the protein content dropped significantly. This assumption is supported by the work of Bhushan *et al* (2016).

The starch contents decreased significantly during storage in both types of bags without biopesticide (control and H0) because of deterioration due to increased insects in stored cowpea. These observations would be linked to the rapid increase in the moisture content of cowpea. The decrease in starch content found in our study is consistent with reports by Nahla (2012), Bhushan *et al* (2016) on infested seeds.

According to them, starch plays an important role in the diets of microorganisms by supplying metabolites necessary for their different life cycles. Simic *et al*. (2007) showed that starches are reduced when exposed to the temperature of 25 ° C for 6 months of storage. In addition, Chattha *et al* (2015) showed the decrease in starch content of wheat grains at 12% moisture during storage in the straw clay bin for 12 months. According to Maréchal and Chrastil (1992), the degradation of proteins and starch can also result from Maillard oxidation reactions.

The total carbohydrates contents have undergone various changes. In polypropylene bags and triple bagging systems without biopesticide, the total carbohydrates contents increased significantly during the storage period whereas in the triple bagging systems with biopesticide, these contents increase gradually after the 7th month of storage. This leads us to assume that the abundant presence of insects in cowpea would increase total carbohydrates contents. The decrease in total and reducing sugars could be due to their consumption by microorganisms for their growth. Indeed according to Olive (2008), after hydrolysis of sugars, microorganisms specifically yeasts would prefer glucose that is directly metabolized. With such a decrease in the main macronutrients the caloric values are logically affected and decrease during storage, as shown by the close correlations between cowpea energy value and protein and lipid contents.

V. CONCLUSION

The aim of this study was to propose to the actors of the cowpea chain in Ivory Coast inexpensive, sustainable technology, protecting the environment and human health, with a view to strengthening cowpea preservation capacities. The results of our study confirm the importance of the establishment of adequate systems for preservation of the protein-energy quality of cowpea seeds. In fact, the triple bagging systems have shown the advantage of extending the shelf life of cowpea seeds. However, the use of triple bagging systems combined with the addition of *Lippia multiflora* leaves as a biopesticide made it possible to preserve the energy-protein quality (macronutrients and energetic value) of cowpea seeds for 8 months. Thus, this biopesticide could therefore be an effective alternative in cowpea preservation as a replacement for synthetic pesticides. The method developed in our study from a biopesticide in the triple bagging systems is inexpensive and promising for Ivorian producers. However, this study needs to be deepened to preserve the micronutrients of the cowpea after storage.

REFERENCES

[1] Agbo N., Soumanou M. and Yao K. (1996). "Nouvelles techniques de conservation de la banane plantain en milieu rural avec de la matière végétale. Sciences des Aliments, vol. **16**, pp 607-621.

[2] Agyen-Sampong, M. (1978). Progress report on investigation on insect of cowpea in Ghana *Tropical Grain Legume Bull*. **8:** 20 -23

[3] Ahenkora K., Adu Dapaah H. K and Agyemang A. (1998). Selected nutritional components and sensory attributes of cowpea (Vigna unguiculata [L] Walp) leaves. *Plant Foods Hum Nutr*., **52 (3)**: 221-229.

[4] AOAC (2000). Official Methods of Analysis of the Association of Analytical Chemists. 17th Edition. Washington, DC, USA.

[5] Archana V. & Jawal N. (2007). Genetic variation and relatedness in vignaunguiculata revealed by microsatellites fonder's day special issue, (N° 285) 190-197.

[6] Aremu D. O, Babajide N. A., Ogunlade C. A., Oyeniran T. and Kadiri A. O. (2015). Effects of storage media and duration on nutritional qualities of cowpea (Vigna unguiculata L. walp). *Journal of Agriculture and Veterinary Science*. **8(1)**: 60-65.

[7] Bambara D. Et Tiemtore J. (2008). Efficacité biopesticide de Hyptis spicigera Lam., Azadirachta indica A. Juss. et Euphorbia balsamifera Ait. sur le niébé *Vigna unguculata* L. Walp., in *Tropicultura*, vol **26** (n°1), 53-55pp.

[8] Baoua I. B., Margam V., Amadou L., Murdock L. L. (2012). Performance of triple bagging hermetic thechnology for postharvest storage of cowpea grain in Niger. *Journal of Stored Products Research,* **51:** 81-85.

[9] Bernfeld D.; "Amylase Banda, In: method in enzymology 1", Colowicck S. P. and Kaplan N. O. (1955). Academic Press, pp 149-154.

[10] Bhushan G., Sharma S. Kr., Dwivedi S., Kumar S., Tandon R. and Singh A. P. (2016). Effect of different storage structure on biochemical alterations in seeds of Penisetum Americanum (Linn.) during storage. *Asian journal of Agriculture & Life Sciences,* **1**(1): 8-14.

[11] Bliss F. A. (1972). Cowpea in Nigeria. In: Milner, M. (ed.) Nutritional Improvement of food Legumes by Breeding. PGA/ FAO. Rome. Pp 151-153.

[12] Boeke J. B., Kossou D. K., Huis A. V., Vanloon J. J. A., Dicke M. (2004). Field trials with plant products to protect stored cowpea against insect damage. *Int. J. Pest Manag*. **50**: 1-9.

[13] Chattha S., Hasfalina C., Lee T., Mirani B et Mahadi M. (2015). A study on the quality of wheat grain stored in straw-clay bin. *Journal of Biodiversity and Environmental Sciences*, **6**: 428-437.

[14] De Groote H., Kimenju S. C., Likhayo P., Kanampiu F., Tefera T., Hellin J. (2013). Effectiveness of hermetic systems in controlling maize storage pest in Kenya. *Journal of Stored Products Research,* **53:** 27-36.

[15] Di Domenico A. S., Christ D., Hashimoto E. H., Busso C. et Coelho S. R. (2015). Evaluation of quality attributes and the incidence of *Fusarium sp*. and *Aspergillus sp*. in different types of maize storage. *Journal of Stored Products Research,* **61**: 59-64.

[16] Doumma A., Salissou O., Sembène M., Sidikou R.S.D., Sanon A., Ketoh G.K., Glitho I.A. (2011). Etude de l'activité reproductrice de Callosobruchus maculatus(F.) (Coleoptera: Bruchidae) sur dix variétés de niébé, Vigna unguiculata(L.) Walp. en présence ou non de son parasitoïde, Dinarmus basalisR. (Hymenoptera: Pteromalidae). *Journal of Animal & Plant Sciences*. Vol. 11, Issue **2**: 1398-1408.

[17] Dubois M., Gilles K., Hamilton J. K., Rebers P. A. and Smith F. (1956). "Colorimetric methods for determination of sugar and related substances", Analytical chemical, pp 350-356 vol. **28**.

[18] FAO (2002). Food energy-methods of analysis and conversion factors. FAO Ed, Rome. 97

[19] Guèye M.T., Dogo S., Jean-Paul W., Georges L. (2011). Lutte Contre Les Ravageurs Des stocks de céréales et de légumineuses au Sénégal et en Afrique occidentale: synthèse bibliographique in Biotechnol. *Agron. Soc. Environ*., 15(1): 183-194.

[20] Houinsou R. L. F., Adjou E. S., Ahoussi E. D., Sahounhloué D. C. K. and Soumanou M. M. (2014). Caractéristiques biochimique et sensorielle du niébé (*Vigna unguiculata*) conservé au moyen des huiles essentielles extraites de plantes de la famille des Myrtaceae. *International Journal of Innovation and Applied Studies*, **9**(1): 428-437.

[21] Huignard J. (1998). Lutte bbiologique contre les Bruchidae, ravageurs du niébé en Afrique de l'ouest, in Agriculture Tropicale Et Subtropicale, 159-164pp.

[22] Ilboudo Z., Dabiré L. C. B., Nébié R. C. H., Dicko I. O., Dugravot S., Cortesero A. M., Sanon A. (2010). Biological activity and persistence of four essential oils towards the main pest of stored cowpeas, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Journal of Stored Products Research* **46:** 124-128.

[23] Isman M. B. (2006). Botanica1 insecticides, deterrents, and repellents in modem agriculture and an increasing1y regu1ated world. *Annual Review of Entomology*, **51**: 45-66.

[24] Jarvis and Walker. (1993). "Simultaneous, rapid, spectrophotometric determination of total starch, amylase and amylopectin", *Journal of the science of Food and Agriculture*, Vol. **63** Issue 1, 53-57.

[25] Kayombo M. A., Mutombo T. J. M., Somue M. A., Muka M. P., Wembonyama O. M., Tshibangu B. K. E., Kaboko K. J. (2014). Effet de la poudre de Basilic (Ocimum basilicum) dans la conservation des grains de Niébé (Vigna *unguiculata* L. Walp.) en stock contre Callosobruchus maculatus F. à MbujiMayi (RD. Congo) in *Congosciences*, vol **2**, n°2, 61-66pp.

[26] Ketoh G. K, Koumaglo H. K. et Glitho I. A. (2005). Inhibition of Callosobruchus maculatus (F.) (Coleoptera:Bruchidae) development with essential oil extracted from Cymbopogonschoenanthus L. Spreng. (Poaceae), and the wasp Dinarmus basalis Rondani (Hymenoptera: Pteromalidae). *J. Stored Prod. Res*., **41**: 363-371.

[27] Kétoh G. K. (1998). Utilisation des huiles essentielles de quelques plantes aromatiques du Togo comme bio pesticides dans la gestion des stades de développement de callosobruchus.

[28] Konan K. C., Fofana I., Coulibaly A., Koffi N. E., Chatigre O., Biego G. H. M. (2016). Optimization of Storage Methods of Cowpea (Vigna Unguiculata L. Walp) Bagged Pics Containing Biopesticide (Lippia Multiflora) By Central Composite Experimental Design in Côte d'Ivoire. *International Journal of Environmental & Agriculture Research* **2**: 46-56.

[29] Konan K. C. (2017). Evaluation de la qualité sanitaire des grains de niébé (vigna unguiculata L. walp.) stockées dans des sacs à triple ensachage en présence d'un biopesticide (Lippia multiflora M.). Thèse de Doctorat d'Etat (Option Biochimie-Sciences des Aliments), UFR Bioscience, Université Félix Houphouet-Boigny, Abidjan, Côte d'Ivoire, 153p

[30] Lange J. & Wyser Y. (2003). Technologie des sciences de l'emballage : Perméabilité à l'oxygène,au dioxyde de carbone et à la vapeur d'eau et transition vitreuse de plusieurs polymères, **16**: 149-158.

[31] Maalekuu B. K. & Kotey E. N. (2014). A survey on methods used in the storage of some varieties of cowpea (*Vigna unguiculata* L) and their effect on quality (A case study in Ejura-Sekyedumase District). *Agriculture and Biology Journal of North America*, **5** (2): 40-50.

[32] Madamba R. (2002). Producing cowpea seed. *The Crop Breeding Institute*.

[33] Marshall W. E and Chrastil J. (1992). Interaction of food protein and starch. In B. J. F. Hudson (Ed.), Biochemistry of food proteins. London, UK: *Elsevier Applied Science*.

[34] Mbah C. E. & Silas B. (2007). Nutrient composition of cowpeas infested with *Callosobruchus maculates* L. in Zaria. *Nigerian Food Journal*, **25**(2): 23-29.

[35] Mukendi R.T., Munyinga K.Y., Nyembo D.N., Ilunga M., Kazadi J.N., Djamba O.E. (2013). Effet de l'huile de palme dans la préservation du niébé en stock contre Callosobrichus maculatusf. (Coleoptera: Bruchidae) en milieu rural de Kabinda (RDC). *Congo Sciences*, vol **1**(1): 9-13.

[36] Mutambuki K., Affognon H., Baribusta D. (2015). Evaluation of triple layer hermetic storage bag (PICS) against *Prostephanus truncates* and *Sitophilus zeamais*. 11th International Working Conference on Stored Product Protection.

[37] Mutungi C., Ng'ang'a J., Imathiu S., Affognon H. (2016). Effect of triple-layer hermetic bagging on mould infection and aflatoxin contamination of maize during multi-month on-farm storage in Kenya. *Journal of Stored Products Research*, **69:** 119-128.

[38] Nahla M. M. H. (2012). Determination of biochemical changes during storage of cowpea infested with the cowpea weevil (Callosobruchus Maculatus F.). *Middle East journal of Applied Sciences* **2 (2)**: 66-70.

[39] Niamketchi L., Chatigre O., Konan Y., Biego G. H (2016). Nutritive compounds evolution of postharvest maize (zea mays l.) stored in granaries with biopesticides from rural conditions in Côte d'Ivoire. *International journal of innovative research in technology & science* **4 (2)** 50-64.

[40] Nteranya S. David B. (2015). Oléagineux et Niébé ; centre international de conférences Abdour Diof ; Sénégal (Dakar) 30 p.

[41] Ojiako F. O. et Kayode R. M. O. (2014). Nutritional quality and consumer acceptability of cowpea seeds (Vigna unguiculata L Walp) treated with natural and synthetic insecticides against bruchid infestation. *Journal of Stored Products Research* **56**: 9-15.

[42] Okunola C. O. et Ofuya T. I. (2007). Effect of some essential plant oilsoninsect infestation of stored maize and cowpea. African crop Science Conference Proceedings, **8**: 1003–1007.

[43] Olive G, 2008. The vitamins. In Biochemistry Volume 2, Industrial and Commercial school of the city of Namur, 172-190. French

[44] Paraginski R., Vanier N., Berrios J., De Oliveira M., Elias M. (2014). Physicochemical and pasting properties of maize as affected by storage temperature. *Journal of Stored Products Research*, **59**: 209-214.

[45] Regnault-Roger C. (2002). De nouveaux phytoinsecticides pour le troisième millénaire ? In: Philogène B.J.R, Regnault-Roger C. & Vincent C., coord. Biopesticides d'origine végétale. Paris: Lavoisier-Éditions Tec & Doc, 19-39.

[46] Rubasinghege G. R. S., Abewickrama K. & Paranagama. (2007). Effect of selected essential oils on physicochemical changes of stored cowpea, *Vigna unguiculata,* treated to control cowpea bruchib, *Callosobruchus maculatus* (F.). pp: 23-24.

[47] Simic B., Popovic R., Sudaric A., Rozman V.,Kalinovic I., Cosic J. (2007). Effect of storage condition on seed oil content of maize, soybean and sunflower. *Agriculturae Conspectus Scientificus*, **72**: 211- 213.

[48] Smart, J. (1964)**.** Pulses in human nutrition, p 96-104 in: Tropical pulses. Longman, London.

[49] Stefanello R., Londero P.M.G., Muniz M.F.B., Alves J.S. and Fischer L. (2015). Chemical composition of landrace maize seeds stored under different conditions. *International Food Research Journal,* **22**: 918-922.

[50] Sule O. S., Emmanuel O. I., Oladipupo D. and Bukola B. O. S. (2014). Effect of *Callosobruchus maculates* Infestation on the Nutrient-antinutrient Composition, Phenolic Composition and Antioxidant Activities of Some Varieties of Cowpeas (*Vigna unguiculata*). *Advance Journal of Food Science and Technology,* **6**(3): 322-332.

[51] Tatsadjieu N. L., Dongmo J. P. M., Ngassoum M. B., Etoa F.-X. & Mbofung C. M. F. (2009). Investigations on the essential oil of *Lippia rugosa* from Cameroon for its potential use as antifungal agent against *Aspergillus flavus* Link ex. Fries. *Food Control,* **20**: 161-166.

[52] Vales M. I., Ranga Rao G. V., Sudini H., Patil S.B, Murdock L. L. (2014). Effective and economic storage of pigeonpea seed in triple layer plastic bags. *Journal of Stored Products Research,* **58:** 29-38.