Use of Triple Bagging System and Lippia Multiflora Leaves for the Mineral Quality Preservation of Cowpea Seeds (Vigna unguiculata L. Walp) and Estimates of Daily Intake

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Abstract - *Cowpea is a food leguminous plant much used in the traditional diet of the populations in Ivory Coast. It is an important source of minerals. However, it is confronted with storage and / or preservation problems which hinder his production in large quantity. This experiment, carried out in Ivory Coast, aimed to evaluate the effectiveness of triple bagging systems with or without Lippia multiflora M. leaves on the preservation of the nutritional quality of cowpea seeds, particularly its mineral potential. Estimation of mineral daily intake was also evaluated at Ivorian adults after 8 months of storage.*

The sampling design for analysis was made according to a 6x6 factorial design. Thus, 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. The second factor, storage time, it included six periods of observation (0; 1; 2; 4.5; 7 and 8 months). Multivariate analysis results (PCA and AHC) indicate that the addition of at least 0.7% Lippia multiflora leaves in triple bagging systems makes preservation more efficient and preserves the mineral quality of the cowpea seeds during 8 months. Estimated intakes are significantly higher when cowpea seeds are stored in the triple bagging systems associated with Lippia multiflora leaves than when the seeds are stored in these same systems without biopesticide.

Key words: **Cowpea, preservation, triples bagging, biopesticide, mineral characteristics, estimated intakes**

1. INTRODUCTION

In many African, Asian and South American countries, cowpea is an integral part of people's traditional diets [1]. Today, with an annual world production of 6.4 million tons [2], cowpea consumption has substantially increased in recent years due to the nutritional profile of seeds (high nutrient content). It is the main source of low cost vegetable protein for vulnerable populations [3]. Cowpea seeds are an important source of micronutrients, including essential minerals that are essential for the proper functioning of the body [4-7]. Due to their relatively interesting mineral composition, cowpea seeds are increasingly used in food programs for local product fortification [8] and in the fight against micronutrient deficiencies [9].

In developing countries, mineral deficiencies remain a public health problem affecting nearly half of the population, particularly children, adolescents and pregnant women [10, 11]. Thus, conventional intervention programs in these regions depend on artificial mineral supplementation [12]. However, many of these programs have proved unsustainable due to their high operating costs [13, 14], distribution problems or access difficulties in the regions [15]. Thus, better use of cowpea seeds in the diets of populations in these areas where nutritional deficiencies are observed could represent a powerful and sustainable intervention tool [14].

Unfortunately, despite its importance, cowpea is faced with storage and / or preservation problems mainly due to attacks caused by pests such as bruchids [16, 17]. 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). These stock pests (bruchids and microorganisms) deteriorate the nutritive value and in turn the mineral composition of seeds stored at the end of storage [7, 18].

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 [19].

Given the extent of the damage caused by the use of these chemicals, the use of biopesticides as an alternative has been encouraged in recent decades [20-22]. Indeed, the use of plants and their derivatives for the protection of food is a very old practice in rural areas. It is an effective means of control, guarantees biodiversity and is less expensive [21, 23-25]. Among the aromatic plants used, appears Lippia multiflora. It is a local and accessible plant in all regions of Ivory Coast whose insecticidal and / or insect repellent properties have been revealed by recent conservation work on cowpea [26-28].

Triple bagging systems that have been shown to be effective in extending the shelf life of cowpea seeds are also frequently used in the preservation of this pulse [29-34]. They consist of a double layer of high density independent polyethylene placed inside a polypropylene woven bag. Thus, the objective of this study is to evaluate the effects of triple bagging systems associated or not with Lippia multiflora leaves (biopesticide) on the mineral composition of cowpea seeds during storage. In addition, an estimate of the nutrient intake of cowpea seeds consumed after 8 months of storage was also evaluated.

2. MATERIAL AND METHODS

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 with average temperature and relative humidity of 28 ± 0.2ºC and 75.0 ± 1.0%, respectively. Wooden pallets have been placed on the floor as a support for the different 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) 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. 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, one adapted inside the other, were enclosed in the polypropylene woven bag.

2.4 Protocol of cowpea seeds preserving

The experiment lasted 8 months. It was implemented using the methodology of preservation by bagging cowpea seeds suggested by [35] 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 [35]. 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.

2.6 Minerals determination

2.6. 1 Samples mineralization

Samples were mineralized in ashes by incineration at 550º C using an electric muffle furnace. Ashes were obtained after incineration of 5 g of samples beforehand carbonized on a Bunsen burner for 12 h until obtaining white residues [36].

2.6. 2 Mineral elements evaluation

The mineral contents of the studied samples were recovered from ashes using an Energy Dispersive Spectrophotometer (EDS), coupled to scanning electron microscope (SEM).

2.6. 3 Operating conditions of the energy dispersion spectrophotometer (EDS)

This device to variable pressure (MEB FEG Supra 40Vp Zeiss) was equipped of an X-ray detector (Oxford instruments) bound to a flat shape of EDS microanalysers (Inca cool dry, without liquid nitrogen). The operative conditions of EDS-SEM were as follows:

- Enlargement: 10x et 1000000x;
- Resolution: 2 nm;
- Variable voltage: 0,1 KeV à 30 KeV ;

The chemical elements were acquired with following parameters: enlargement, 50x; probe diameter, 30 nm to 120 nm; probe energy, 20 KeV and 25 KeV; working distance (WD), 8.5 mm.

2.6. 4 Validation test of the mineral determination method

The minerals analysis method has been validated according to standard procedure [37, 38] which consists in determination of the linearity, repeatability, reproducibility, extraction yields, detection and quantification limits.

The linearity of 9 mineral elements was tested between 25% and 125% using 5 standard points (25%, 50%, 75%, 100% and 125%).

The repeatability and reproducibility tests were achieved with standards of the different minerals at a concentration of 25%.

Thirty (30) tests were performed respectively for the repeatability and reproducibility tests.

Additions of 5% of the standards have been achieved for the determination of mineral extraction yields. Ten (10) separate tests were conducted for the proportions added.

2.7 Contribution estimated in essential minerals from consumption of the studied cowpea seeds

The mineral intakes have been estimated according to the method of the Codex Alimentarius that takes into account the mineral concentration found in the food (cowpea seeds stored for 8 months) and the daily consumption of an adult of 70 kg t in Ivory Coast according to the following formula [39]:

DI = C x Q

With, DI: daily intake; C: mean concentration; Q: daily food consumption (with is 4.93 g/j of cowpea seed in Ivory Coast [40]. Thus, the contribution deriving from the daily intake on the daily recommended intake basis was determined as below [41]:

Contribution (%) = (DI x 100) / DRI

With, DI: daily intake; DRI: daily recommended intake

2.7 Statistical Analysis

The statistical analyses of the data were carried out thanks to software SPSS (version 22.0) and STATISTICA (version 7.1). All tests related to the mineral characteristics analyzes were performed in triplicate and the results are expressed as mean ± standard deviation. An analysis of variance (repeated measures ANOVA) 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 storage period (7 and 8 months). The significant differences were highlighted by the Tukey post-hoc test at 5% threshold. Finally, Multivariate Statistical Analysis (MSA) including Principal Component Analysis (PCA) and Hierarchical Ascending Clustering Analysis (HAC) were performed to classify samples with similar behavior across all minerals over storage time.

3. RESULTS

3.1 Validation parameters for quantification of minerals using EDS.

The results of the validation tests are presented in Table 1. The determination coefficient (R^2) recovered from the standard lines are included between 0.99 and 1.

The minerals limits of detection vary from 104 µg/kg to 581 µg/kg and their minimal values quantified are between 146 µg/kg and 796 µg/kg.

The coefficients of variation (CV) from 10 repeatability tests oscillate between 1.0% and 1.8%, while 15 reproducibility tests result in CV 2.3% to 4.7%. These results translate stability and a satisfactory precision of microanalysis technics.

About the minerals added, the extraction yields run from 97.3% to 99.5%, revealing minerals extraction defaults between 0.5% and 2.7%. The method is reliable and exact.

3.2 Evolution of mineral contents of cowpea seeds according to treatments during preservation

Statistical test data used to evaluate all minerals during storage are shown in Tables 2 and 3. The tests carried out reveal significant variations ($P < 0.001$) in the mineral contents according to the duration and type of treatments (triple bagging and / or biopesticide). In addition, the interaction between these two variables has a significant effect (Table 2).

3.2. 1 Macroelements contents of cowpea seeds

Potassium (K), phosphorus (P), magnesium (Mg), sodium (Na) and calcium (Ca) are the five major elements that have been identified in stored cowpea seeds (Table 4). The results show that the mineral contents change differently in cowpea seeds during the 4.5 months storage period for the polypropylene control bag (TST); 8 months for triple bagging without biopesticide (H0) and four triple bagging systems with different proportions of biopesticide (H1: 0.7%, H2: 2.5%, H3: 4.3% and H4: 5%). With an average of 13.14 ± 0.01 g / kg at the storage began (month 0), the K content decreases significantly ($P < 0.001$) in the polypropylene control (TST) to reach a value of 11, 01 ± 0.01 g / kg during the 4.5 months of storage, then in the triple bagging system without biopesticide (12.07 ± 0.01 g / kg) after 8 months of storage. However, in four triple bagging systems whatever the proportion of biopesticide, these values remain statistically identical ($P > 0.05$) with an average of 13.02 ± 0.02 g / kg after 8 months of storage (fig. 1).

Mg and Na contents in the control (TST) dropped significantly ($P < 0.001$) during the 4.5 months of storage from 1.37 ± 0.02 g / kg to 0.43 ± 0.01 g / kg and from 0.69 ± 0.01 g / kg to 0.36 ± 0.01 g / kg respectively for magnesium and sodium. In the triple bagging system without biopesticide (H0), after 8 months of storage, these values went from 1.37 ± 0.02 g / kg to 0.49 ± 0.01 g / kg for Mg and 0.69 ± 0.01 g / kg to 0.40 ± 0.01 g / kg for Na (fig. 1). The average Mg and Na contents in triple bagging systems with different proportions of biopesticide are respectively in the order to 1.02 ± 0.01 g / kg and 0.53 ± 0.01 g / kg at the end of the 8 month of preservation (fig. 1).

Concerning P, the lowest contents were recorded after 4.5 months of storage in the TST control (values decreased from 2.31 ± 0.01 g / kg to 1.38 ± 0.01 g / kg) and 8 months of storage in H0 (values decreased from 2.31 ± 0.01 g / kg to 1.66 ± 0.02 g / kg). The P content of the cowpea seeds in H4 batch after 8 months of storage remains higher than the other values of the samples preserved with biopesticide. It is 2.22 ± 0.04 g / kg, whereas the P contents for H1, H2 and H3 are respectively to 1.85 ± 0.02 g / kg, 1.90 ± 0.10 g / kg and 1.98 ± 0.16 g / kg (fig. 1).

For Ca, the content of the control samples (TST) decreased from 0.59 ± 0.01 g / kg to 0.29 ± 0.01 g / kg during 4.5 months of possible storage. A similar variation was made in the triple bagging system without biopesticide (H0), which in 8 months of storage decreased from 0.59 ± 0.01 g / kg to 0.40 ± 0.01 g / kg (fig. 1). In the triple bagging systems associated with doses of biopesticide, the Ca content of cowpea seed after 8 months of storage are on average to 0.47 ± 0.01 g / kg, 0.48 ± 0.01 g / kg, 0.50 ± 0.01 g / kg and 0.51 ± 0.02 g / kg respectively for H1, H2, H3 and H4 (fig. 1).

3.2. 2 Oligoelements contents of cowpea seeds

During storage, iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) are the four oligoelements that have been identified in cowpea seeds (Table 5). Before storage, the Cu and Mn contents in the seeds were 26.53 ± 0.51 mg / kg and 36.03 ± 0.12 mg / kg, respectively. These contents decreased significantly ($P < 0.001$) during the first 4.5 months of storage in the polypropylene control at values of 14.73 ± 0.38 mg / kg for Cu and 17.44 ± 1, 09 mg / kg for the Mn. After 8 months of storage, a similar decrease in Cu and Mn contents was observed with respective values of 15.57 ± 2.06 mg / kg and 19.17 ± 0.95 mg / kg in triple bagging without biopesticide (fig. 2). However, after 8 months of storage, the one-way ANOVA showed no statistically significant difference (P> 0.05) between samples stored in triple bagging systems with different proportions of biopesticide for these two minerals contents above-mentioned.

Fe and Zn contents in the polypropylene control group respectively decreased from 134.69 ± 5.06 mg / kg to 63.16 ± 0.65 mg / kg and 56.10 ± 0.20 mg / kg to 24.13 ± 0.97 mg / kg during the 4.5 months of storage (Table 5). Similar observations were made in the triple bagging system without biopesticide (H0) that in 8 months of storage recorded contents ranging from 134.69 ± 5.06 mg / kg to 77.87 ± 1.43 mg / kg for Fe and 56.10 ± 0.20 mg / kg to 29.80 ± 1.05 mg / kg for Zn (fig. 2). The Fe and Zn contents after 8 months of storage in the triple bagging systems associated with the biopesticide remain well above the contents of the TST and H0 batches with averages of 110.70 ± 0.50 mg / kg, 121.73 ± 2.10 mg / kg, 127.50 ± 1.71 mg / kg, 128.90 ± 1.01 mg / kg for Fe and 44.70 ± 0.53 mg / kg, 49.43 ± 1.15 mg / kg, 51.68 ± 0.46 mg / kg, 52.03 ± 0.81 mg / kg for Zn respectively in H1, H2, H3 and H4.

3.3 Multivariate Analysis

The Principal component analysis (PCA) was performed with the F1 and F2 factors, which support 95.08% of the total variability. F1 component records eigenvalue of 8.31 and expresses 92.32% variance, and factor F2 accounts eigenvalue of 0.25 for only 2.76% of variance (fig. 3a).

Fig. 3b shows the variability (gatherings) of the studied samples. It divides the samples into 3 groups. Group 1 consists of individuals from the triple bagging system without biopesticide (H0) at 7 and 8 months of storage (noted B4 and B5) and polypropylene control (TST) at 2 and 4.5 months of storage (noted A2 and A3). These samples are characterized by the lowest values of minerals contents. The second group consists of three individuals from the triple bagging system without biopesticide at 1; 2 and 4.5 months of storage (B1, B2 and B3) and an individual polypropylene bag (control) at 1 month storage (A1). Their mineral content is higher than that of group 1 but significantly lower than the other samples. Group 3 contains all cowpea samples resulting from triple bagging systems with biopesticide at all retention periods as well as the initial sample (Ei). This group is distinguished by the highest values for all minerals.

The Ascending hierarchical classification (AHC) corroborates the variability observed in the PCA (fig. 4). Indeed, at aggregation distance of 64, the dendrogram shows three clusters of cowpea samples during storage. The first cluster consists of 4 individuals (B4, A3, B5 and A2) with lowest values of minerals contents. The second cluster encloses three individuals resulting from the triple bagging system without biopesticide (B1, B2 and B3) and 1 individual from polypropylene bag (A1). These individuals are distinguished by their mineral content which is higher than that of first cluster but significantly lower than the other samples. The third cluster includes all cowpea samples resulting from triple bagging systems with biopesticide at all retention periods and the initial sample (Ei). Individuals in the latter cluster have the highest values for minerals contents.

3.4 Estimated daily intakes and contribution of essential minerals

Table 6 shows the essential minerals estimated intakes from the consumption of cowpea seed stored for 8 months.

The total potassium and magnesium intake of samples stored in the triple bagging systems with biopesticide remain in the order of 64.19 mg / day and 5.03 mg / day, respectively. On the other hand, in the triple bagging system without biopesticide (H0), the estimated intake of these minerals is 59.50 mg / day for potassium and 2.42 mg / day for magnesium. The total phosphorus intake ingested by an adult is 8.18 mg / day for H0 and varies between 9.12 mg / day and 10.94 mg / day in triple bagging systems with biopesticide. The daily contribution in sodium ingested is 1.97 mg / day in H0 and 2.56 mg / day; 2.61 mg / day; 2.61 mg / day and 2.66 mg / day respectively in H1, H2, H3 and H4. Calcium daily intake is 1.97 mg / day; 2.32 mg / day; 2.37 mg / day; 2.47 mg / day and 2.51 mg / day respectively in H0, H1, H2, H3 and H4. Regarding oligoelements, iron intake is highest with an average value of 0.60 mg / day in triple bagging systems with biopesticide. In H0 this intake is 0.38 mg / day. The zinc estimated intake is between 0.22 mg / day and 0.26 mg / day in the triple bagging systems with biopesticide, whereas in the triple bagging system without biopesticide this contribution is 0.15 mg / day. Manganese and copper have respective daily intakes of 0.15 mg / day and 0.11 mg / day in triple bagging systems with biopesticide. In H0, these intakes are 0.09 mg / day for Mn and 0.08 mg / day for Cu.

In terms of contribution, cowpea seeds stored in triple bagging systems with biopesticide provide the highest share (0.10% to 3.21%) in macroelements. Also, at the oligoelements level such as copper (11.14% to 11.67%) and manganese (7.51% to 7.98%). For iron (3.90% to 4.54%) and zinc (2.20 to 2.56%) of the daily recommended intake would be covered.
On the other hand, in the triple bagging system without biopesticide, the contribution in macroelements varies between 0.08% and 2.97%, those for oligoelements is between 1.47% and 7.68% (Table 7).

Estimates of the mineral contributions the samples stored in the polypropylene bag (control) have not been assessed because its shelf life could not reach 8 months. At 4.5 months of storage pests have created significant damage such as market quality so it was removed from storage.

Table 1
Data from validation parameters for evaluation of minerals contents using the energy diffusion spectrometer (EDS).

Mineral	Linearity		CV Repeat. (%. n= 10)	CV Reprod. (%. n= 15)	Ext yield (%. n= 10)	LOD (µg/kg)	LOQ (µg/kg)
	Standard	CD (R^2)					
Fe	Y= 2285x - 88	0.99	1.4±0.07	3.6±0.01	99.5±0.17	107±0.32	149±0.55
Zn	Y= 4365x - 523	0.99	1.3±0.51	3.2±0.96	98.3±0.03	281±0.58	396±0.29
Mg	1452x + 237	0.99	1.1±0.21	3.1±1.44	97.9±0.68	426±0.11	635±0.19
Cu	1953x + 6951	0.99	1.8±0.95	2.5±0.03	98.8±0.43	104±0.05	146±0.63
P	2667x + 1742	0.99	1.4±0.11	3.7±1.22	99.4±0.66	334±0.21	467±0.88
K	3821x + 3838	1	1.3±0.04	4.7±0.32	98.4±1.51	581±0.04	796±0.09
Na	2083x + 147	0.99	1.2±0.05	3.4±0.48	98.8±0.33	261±0.74	365±0.07
Ca	6581x + 5287	1	1.5±0.43	2.3±0.93	97.3±0.84	514±0.15	704±0.47
Mn	3659x + 74454	1	1.2±1.01	2.9±0.77	99.0±0.78	337±0.81	488±0.60

CD, coefficient of determination; **CV Repeat**, coefficient of variation from repeatability test; **CV Reprod**, coefficient of variation from reproducibility test; **Ext yield**, extraction yield from added minerals; **LOD**, limit of detection; **LOQ**, limit of quantification; **K**, potassium ; **P**, phosphorous ; **Mg**, magnesium ; **Na**, Sodium ; **Ca**, Calcium ; **Fe**, iron ; **Zn**, Zinc ; **Mn**, manganese ; **Cu**, copper

Table 2
Statistical Data (Repeated Measure ANOVA) of Cowpea mineral levels under treatment during preservation

SOV	Stat Para.	K	P	Mg	Na	Ca	Fe	Zn	Mn	Cu
Durations	**df**	1.71	3	3	3	3	3	3	3	3
	SS	2.27	0.92	1.13	0.26	0.12	5825.98	1368.18	439.30	176.88
	F	718.685	477.62	1.45	751.52	553.88	183.75	396.62	180.29	349.65
	P					**< 0.001**				
Error	**ddl**	20.46	36	36	36	36	36	36	36	36
	SC	0.04	0.02	9.34	0.00	0.00	380.48	41.41	29.24	6.07
Treatments	**df**	5	5	5	5	5	5	5	5	5
	SS	14.43	3.20	1839.77	0.28	0.27	18902.58	3699.71	816.55	476.11
	F	3335.29	441.85	0.64	574.75	648.40	278.85	362.35	156.85	432.32
	P					**< 0.001**				
Error	**df**	12	12	12	12	12	12	12	12	12
	SS	0.01	0.02	6.92	0.00	0.00	162.69	24.51	12.49	2.64
Durations x Treatments	**df**	8.52	15	15	15	15	15	15	15	15
	SS	6.12	1.18	975.36	0.10	0.10	6994.61	1293.69	399.77	179.54
	F	387.24	123.13	0.25	59.77	95.35	44.12	75.01	32.81	70.98
	P					**< 0.001**				

SOV, source of variation; **Stat Para**, statistical parameters; **df**, degree of freedom; **SS**, sum of squares; **F**, value of the statistical test; **P**, probability value of the statistical test, **K**, potassium ; **P**, phosphorous ; **Mg**, magnesium ; **Na**, Sodium ; **Ca**, Calcium ; **Fe**, iron ; **Zn**, Zinc ; **Mn**, manganese ; **Cu**, copper.

Table 3
Statistical Data (ANOVA 1) of Cowpea mineral levels under treatment during preservation

Effect	Stat para	K	P	Mg	Na	Ca	Fe	Zn	Mn	Cu
Treatments	df	4	4	4	4	4	4	4	4	4
	SS	2.159	0.511	694.363	0.040	0.021	5335.2	1030.54	364.87	139.703
	F	68.10	16.795	440.16	65.59	53.43	626.98	358.57	58.673	35.255
	P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.05**	**<0.001**
Error	df	10	10	10	10	10	10	10	10	10
	SS	0.079	0.076	3.944	0.001	0.001	21.3	7.18	15.55	9.907
Total	df	14	14	14	14	14	14	14	14	14
	SS	2.238	0.587	698.307	0.042	0.022	5356.5	1037.73	380.42	149.609

Stat Para, statistical parameters; **df**, degree of freedom; **SS**, sum of squares; **F**, value of the statistical test; **P**, probability value of the statistical test, **K**, potassium ; **P**, phosphorous ; **Mg**, magnesium ; **Na**, Sodium ; **Ca**, Calcium ; **Fe**, iron ; **Zn**, Zinc ; **Mn**, manganese ; **Cu**, copp*er*.

Table 4
Evolution of macroelements contents of cowpea seeds preserved according to different treatments for 4.5 months

Minerals	Storage time (month)	TST	H0	H1	H2	H3	H4
K (g/kg)	0	13.14±0.01^{aA}	13.14±0.01^{aA}	13.14±0.01^{aA}	13.14±0.01^{aA}	13.14±0.01^{aA}	13.14±0.01^{aA}
	1	12.04±0.06^{bC}	12.97±0.05^{bB}	13.07±0.03^{bA}	13.10±0.01^{aA}	13.14±0.01^{aA}	13.14±0.01^{aA}
	2	11.35±0.01^{cC}	12.93±0.06^{bB}	13.06±0.02^{bA}	13.09±0.02^{abA}	13.12±0.03^{aA}	13.13±0.03^{aA}
	4.5	11.01±0.01^{dC}	12.62±0.10^{cB}	13.05±0.01^{bA}	13.08±0.01^{abA}	13.10±0.02^{aA}	13.11±0.01^{aA}
P (g/kg)	0	2.31±0.01^{aA}	2.31±0.01^{aA}	2.31±0.01^{aA}	2.31±0.01^{aA}	2.31±0.01^{aA}	2.31±0.01^{aA}
	1	1.71±0.07^{bC}	1.82±0.04^{bB}	2.24±0.02^{bAB}	2.26±0.01^{aAB}	2.29±0.01^{aA}	2.31±0.01^{aA}
	2	1.58±0.09^{cC}	1.79±0.01^{cB}	2.23±0.02^{bAB}	2.26±0.02^{aAB}	2.28±0.01^{aA}	2.29±0.01^{aA}
	4.5	1.38±0.01^{dD}	1.78±0.02^{cC}	2.11±0.06^{cB}	2.24±0.02^{aAB}	2.25±0.02^{aA}	2.28±0.01^{aA}
Mg (g/kg)	0	1.37±0.02^{aA}	1.37±0.02^{aA}	1.37±0.02^{aA}	1.37±0.02^{aA}	1.37±0.02^{aA}	1.37±0.02^{aA}
	1	0.99±0.01^{bC}	1.01±0.01^{bC}	1.17±0.02^{bB}	1.32±0.01^{aA}	1.33±0.01^{aA}	1.34±0.01^{aA}
	2	0.72±0.07^{cD}	1.01±0.01^{bC}	1.13±0.00^{cB}	1.31±0.00^{aA}	1.33±0.01^{aA}	1.33±0.00^{aA}
	4.5	0.43±0.01^{dD}	0.99±0.01^{bC}	1.03±0.00^{dB}	1.04±0.00^{bB}	1.30±0.01^{aA}	1.31±0.01^{aA}
Na (g/kg)	0	0.69±0.01^{aA}	0.69±0.01^{aA}	0.69±0.01^{aA}	0.69±0.01^{aA}	0.69±0.01^{aA}	0.69±0.01^{aA}
	1	0.50±0.01^{bB}	0.53±0.02^{bB}	0.65±0.01^{bAB}	0.66±0.01^{bAB}	0.68±0.01^{aA}	0.68±0.01^{aA}
	2	0.41±0.01^{cC}	0.51±0.01^{bB}	0.62±0.01^{bAB}	0.63±0.01^{bAB}	0.64±0.01^{bA}	0.65±0.01^{bA}
	4.5	0.36±0.01^{dD}	0.46±0.02^{cC}	0.57±0.01^{cB}	0.60±0.02^{cAB}	0.60±0.01^{cAB}	0.61±0.02^{cA}
Ca (g/kg)	0	0.59±0.01^{aA}	0.59±0.01^{aA}	0.59±0.01^{aA}	0.59±0.01^{aA}	0.59±0.01^{aA}	0.59±0.01^{aA}
	1	0.41±0.00^{bE}	0.48±0.01^{bD}	0.54±0.01^{bC}	0.57±0.01^{abB}	0.58±0.01^{aA}	0.59±0.01^{aA}
	2	0.32±0.02^{cD}	0.47±0.01^{bC}	0.54±0.01^{bB}	0.55±0.01^{bB}	0.57±0.01^{aA}	0.57±0.01^{aA}
	4.5	0.29±0.01^{dD}	0.45±0.01^{cC}	0.51±0.02^{cB}	0.52±0.01^{cB}	0.55±0.01^{bA}	0.56±0.02^{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).

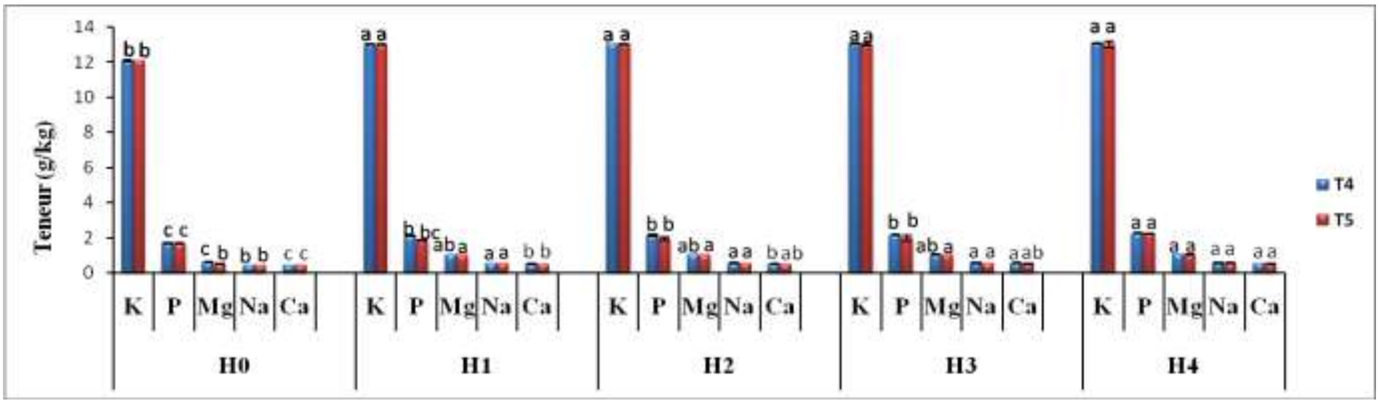


Figure 1: Macroelements content of cowpea seeds stored for 7 and 8 months according to different treatments

Note: histograms with the same letter for each of the minerals according to the treatment are not significantly different at t $P > 0.05$. With **T4**: content after 7 months of preservation; **T5**: content after 8 months of storage; **H0**: Triple bagging without biopesticide; **H1**, **H2**, **H3**, **H4**: Triple bagging with respectively (0.7%, 2.5%, 4.3% and 5%) of biopesticide (w / w).

Table 5
Evolution of oligoelements contents of cowpea seeds preserved according to different treatments for 4.5 months

Minerals	Storage time (month)	TST	H0	H1	H2	H3	H4
Fe (mg/kg)	0	134.69±5.06^{aA}	134.69±5.06^{aA}	134.69±5.06^{aA}	134.69±5.06^{aA}	134.69±5.06^{aA}	134.69±5.06^{aA}
	1	89.20±1.73^{bD}	100.47±1.39^{bC}	128.40±1.47^{abB}	130.13±0.85^{aB}	133.27±0.47^{aA}	133.77±1.43^{aA}
	2	73.03±1.36^{cD}	96.37±6.33^{bC}	125.40±0.61^{bB}	129.15±1.65^{abAB}	132.33±1.53^{abA}	132.70±2.12^{aA}
	4.5	63.16±0.65^{dD}	94.50±5.03^{bC}	118.00±4.53^{cB}	126.13±0.95^{bAB}	131.90±0.82^{abA}	132.47±2.90^{aA}
Zn (mg/kg)	0	56.10±0.20^{aA}	56.10±0.20^{aA}	56.10±0.20^{aA}	56.10±0.20^{aA}	56.10±0.20^{aA}	56.10±0.20^{aA}
	1	32.57±1.55^{bD}	42.43±2.52^{bC}	48.80±1.23^{bB}	54.73±2.06^{aA}	55.53±1.48^{aA}	55.57±1.10^{aA}
	2	29.80±1.47^{cD}	39.50±1.57^{cC}	47.73±0.96^{bcB}	53.70±2.23^{abcA}	54.83±0.64^{aA}	55.43±0.68^{aA}
	4.5	24.13±0.97^{dD}	36.73±0.57^{dC}	46.70±1.31^{cB}	52.07±0.21^{bAB}	52.67±0.15^{bAB}	54.60±0.61^{aA}
Mn (mg/kg)	0	36.03±0.12^{aA}	36.03±0.12^{aA}	36.03±0.12^{aA}	36.03±0.12^{aA}	36.03±0.12^{aA}	36.03±0.12^{aA}
	1	28.90±1.59^{bC}	31.20±0.98^{bB}	33.80±1.04^{bAB}	34.20±0.95^{bAB}	35.67±0.40^{aA}	35.97±1.36^{aA}
	2	21.00±1.01^{cD}	28.73±0.50^{cC}	31.70±2.17^{cB}	33.50±0.61^{bAB}	35.30±0.35^{aA}	35.43±0.58^{aA}
	4.5	17.44±1.09^{dE}	26.60±1.31^{dD}	31.40±1.13^{cC}	32.70±0.40^{bBC}	33.93±1.06^{abAB}	35.03±1.01^{aA}
Cu (mg/kg)	0	26.53±0.51^{aA}	26.53±0.51^{aA}	26.53±0.51^{aA}	26.53±0.51^{aA}	26.53±0.51^{aA}	26.53±0.51^{aA}
	1	19.37±0.12^{bD}	20.47±0.25^{bC}	24.37±0.38^{bB}	25.90±0.02^{bA}	26.33±0.32^{aA}	26.33±0.31^{aA}
	2	17.67±0.68^{cE}	19.53±0.40^{cD}	23.87±0.06^{cC}	25.20±0.36^{bB}	26.23±0.15^{aA}	26.33±0.15^{aA}
	4.5	14.73±0.38^{dD}	19.03±0.81^{cC}	23.80±0.82^{cB}	24.97±0.23^{cA}	25.80±0.10^{aA}	25.93±0.15^{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).

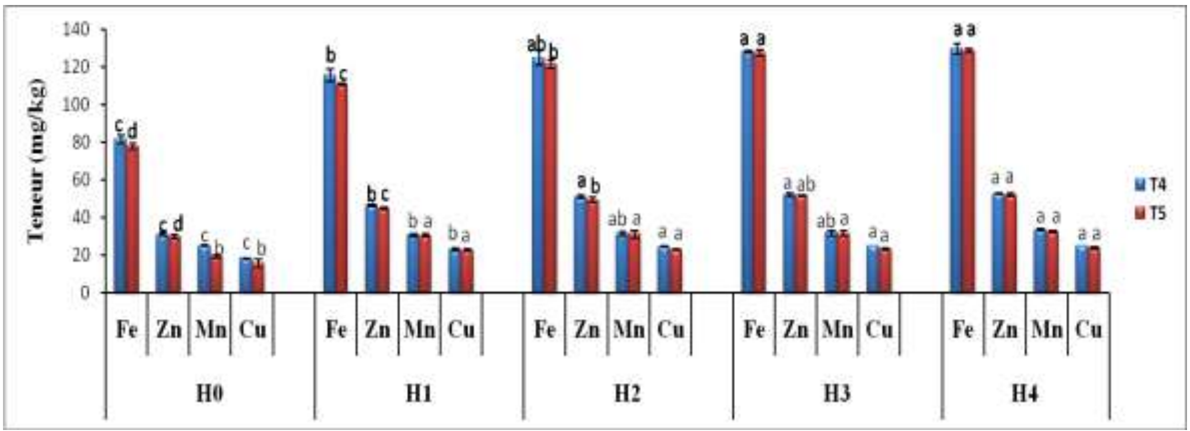
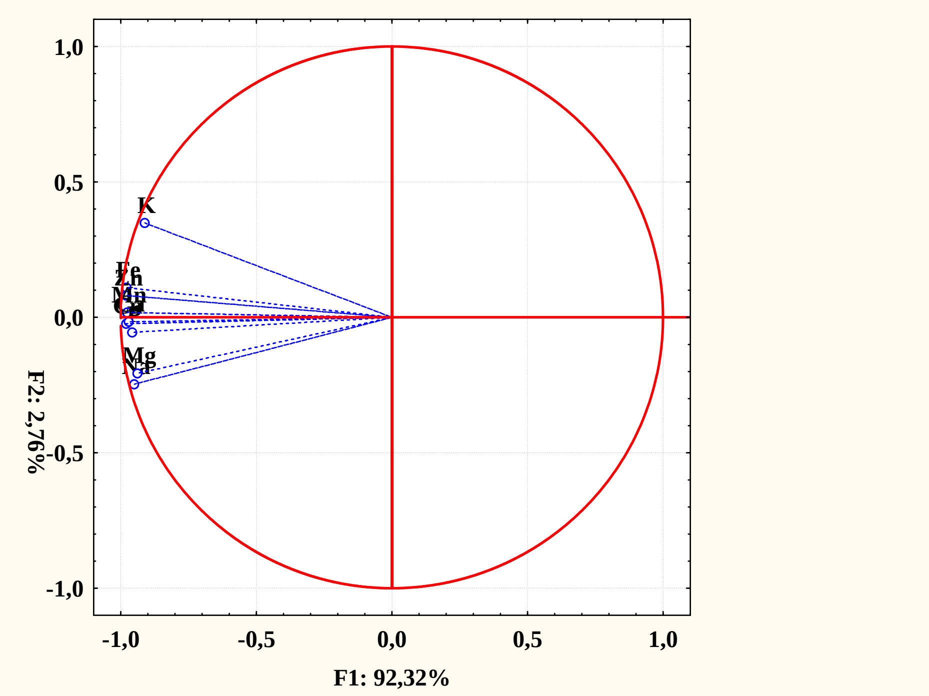
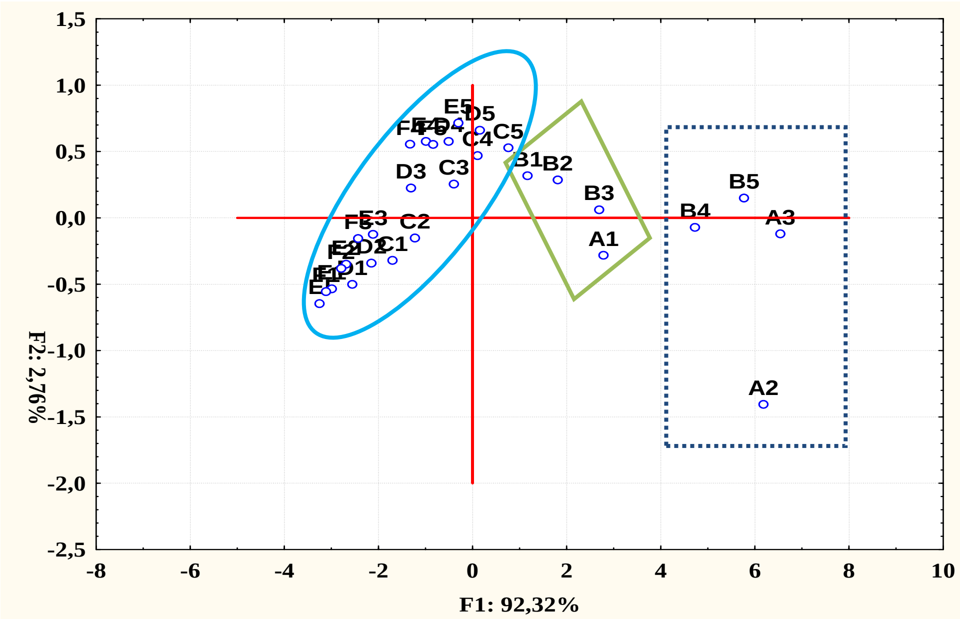


Figure 2: Oligoelements content of cowpea seeds stored for 7 and 8 months according to different treatments

Note: histograms with the same letter for each of the minerals according to the treatment are not significantly different at t P > 0.05. With **T4**: content after 7 months of preservation; **T5**: content after 8 months of storage; **H0**: Triple bagging without biopesticide; **H1**, **H2**, **H3**, **H4**: Triple bagging with respectively (0.7%, 2.5%, 4.3% and 5%) of biopesticide (w / w).



(a)



(b)

Figure 3: Correlation drawn between the F1-F2 factorial design of the principal component analysis and the mineral characteristics (a) and Individuals (b) deriving from the cowpea samples studied.

K, potassium ; **P**, phosphorous ; **Mg**, magnesium ; **Na**, Sodium ; **Ca**, Calcium ; **Fe**, iron ; **Zn**, Zinc ; **Mn**, manganese ; **Cu**, copper; **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**, and **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**, and **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**, and **F5**: triple bagging with 0.7%, 2.5%, 4.3% and 5% biopesticide at 8 months of storage.

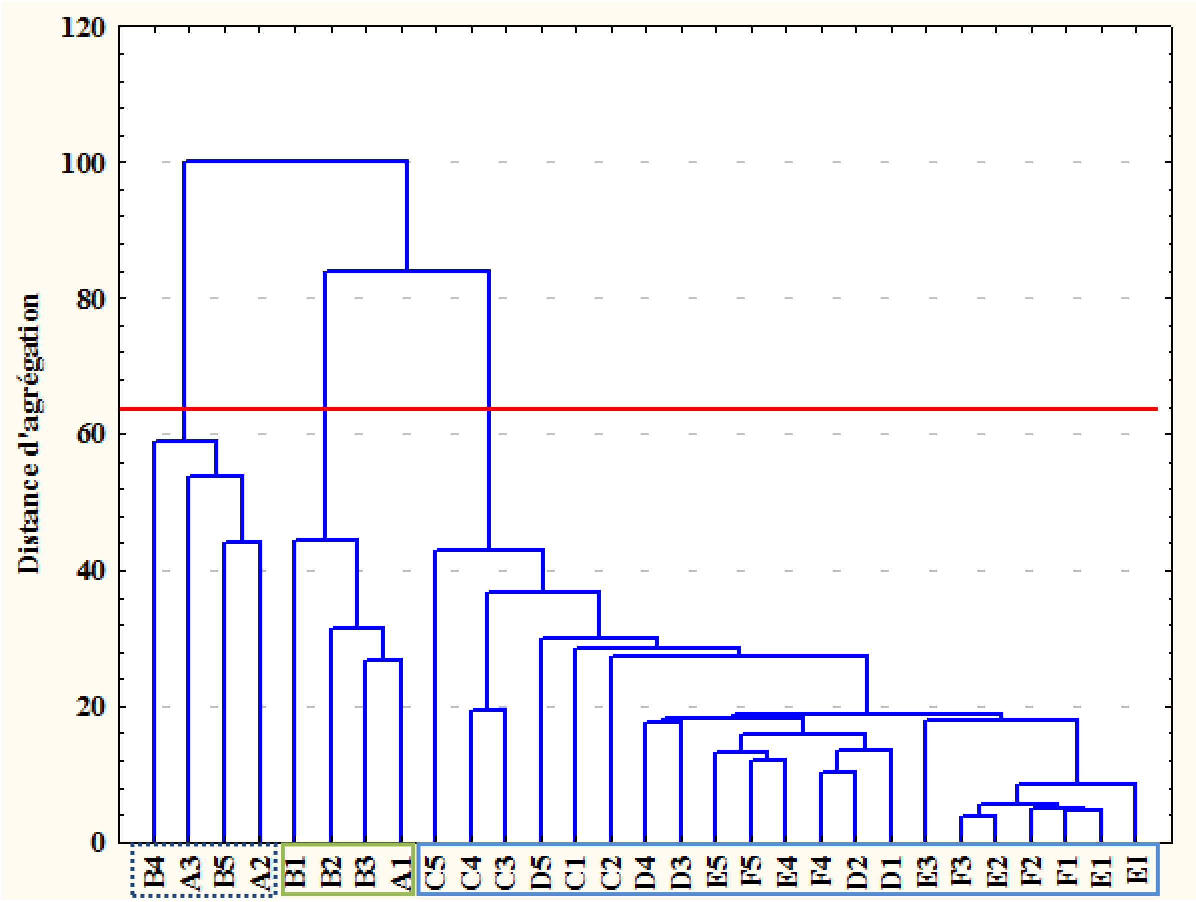


Figure 4: Ascending hierarchical clustering (dendrogram) with the mineral characteristics of cowpea seeds preserved according to different treatments

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**, and **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**, and **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**, and **F5**: triple bagging with 0.7%, 2.5%, 4.3% and 5% biopesticide at 8 months of storage.

Table 6: Estimated daily intakes of essential minerals from cowpea seeds after 8 months of storage according to level of consumption

	Macroelements (mg/day)										Oligoelements (mg/day)							
Samples	K		P		Mg		Na		Ca		Fe		Zn		Mn		Cu	
	DRI	DI	DRI	DI	DRI	DI	DRI	DI	DRI	DI	DRI	DI	DRI	DI	DRI	DI	DRI	DI
H0		59.50		8.18		2.42		1.97		1.97		0.38		0.15		0.09		0.08
H1		64.14		9.12		5.03		2.56		2.32		0.55		0.22		0.15		0.11
H2	2000	64.19	700	9.34	375	5.03	2500	2.61	800	2.37	14	0.60	10	0.24	2	0.15	1	0.11
H3		64.19		9.76		5.03		2.61		2.47		0.63		0.25		0.16		0.11
H4		64.19		10.94		5.08		2.66		2.51		0.64		0.26		0.16		0.12

DRI: daily recommended intake (mg/day); **DI**: daily intakes (mg/day); **H0**: Triple bagging without biopesticide; **H1**, **H2**, **H3**, **H4**: Triple bagging with respectively (0.7%, 2.5%, 4.3% and 5%) of biopesticide (w / w); **K**, potassium ; **P**, phosphorous ; **Mg**, magnesium ; **Na**, Sodium ; **Ca**, Calcium ; **Fe**, iron ; **Zn**, Zinc; **Mn**, manganese; **Cu**, copper.

Table 7: Contribution of cowpea seeds after 8 months of storage to the satisfaction of daily minerals recommended intakes

Samples	Macroelements					Oligoelements			
	K	P	Mg	Na	Ca	Fe	Zn	Mn	Cu
	Contribution (%)	Contribution (%)	Contribution (%)	Contribution (%)	Contribution (%)	Contribution (%)	Contribution (%)	Contribution (%)	Contribution (%)
H0	2.97	1.17	0.64	0.08	0.25	2.74	1.47	4.72	7.68
H1	3.21	1.30	1.34	0.10	0.29	3.90	2.20	7.51	11.14
H2	3.21	1.34	1.34	0.10	0.30	4.29	2.44	7.68	11.30
H3	3.21	1.39	1.34	0.10	0.31	4.49	2.55	7.79	11.52
H4	3.21	1.56	1.35	0.11	0.31	4.54	2.56	7.98	11.67

H0: Triple bagging without biopesticide; **H1**, **H2**, **H3**, **H4**: Triple bagging with respectively (0.7%, 2.5%, 4.3% and 5%) of biopesticide (w / w); **K**, potassium ; **P**, phosphorous ; **Mg**, magnesium ; **Na**, Sodium ; **Ca**, Calcium ; **Fe**, iron ; **Zn**, Zinc ; **Mn**, manganese ; **Cu**, copper.

4. DISCUSSION

4.1 Validation parameters

The R^2 determination coefficients obtained from the calibrations tests were close to 1 (Table 1), thus reflecting a quasi-linear estimation of the different minerals according to their concentration from the samples. In addition, the low coefficients of variation (<5%) resulting from reproducibility and repetition fully reflect the stability of the energy dispersive spectrophotometer (EDS) method used, which is as adjusted since the full amount of each mineral element is indicated, as shown by the low extraction defects below 2.7% from the added minerals. Thus, these characteristics highlight the reliability and precision of the results in the determination of mineral contents using the EDS method.

4.2 Minerals contents

Duration and preservation method are some very important factors affecting the quantity and the nutritional quality of stored cowpea seeds. In order to get a general idea on the nutritional quality and especially the preservation and estimated contribution of different minerals of cowpea seeds during storage, analyzes were carried out during all sampling periods. Minerals considered as essential nutrients for life form the main electrolyte of the human body, maintain tissue homeostasis and at the same time form the main structural component of bones and teeth. However, under uncontrolled conditions of storage / preservation of cowpea seeds, these minerals undergo modifications [42, 43]. In general, the results of this study show that the preservation technique using Lippia multiflora leaves is effective in preserving the mineral quality of cowpea seeds during storage. Indeed, for all the minerals, the highest contents were recorded in the triple bagging systems added to the Lippia multiflora leaves compared to the triple bagging system without biopesticide and the control bag (polypropylene) which record the lowest values at the end of storage.

In the triple bagging systems without biopesticide, mineral elements are preserved over a period of 4.5 months. This preservation of mineral composition would be due to the low oxygen levels in these systems. During storage of cowpea, low oxygen levels in triple bagging inhibit the development of seed-borne insects, cause cessation of their feeding activities, and therefore to reduce damage [44-46]. The significant decrease in mineral contents of cowpea seeds at 7 and 8 months in the triple bagging system without biopesticide and the polypropylene bag (control) to 4.5 months of storage could be explained by the increased metabolic activities of insect populations. These results are consistent with the finding of [7]. Indeed, according to [18, 42, 47], insect pests during their different stages of development would use a large amount of minerals as nutrients necessary for the maintenance of life and to accomplish larval development and metamorphosis. In contrast, Lippia multiflora have insecticidal and repellent effects that result in the maintenance of seed mineral content in the triple bagging systems with biopesticide. The anti-palatability effect of Lippia multiflora on Callosobruchus maculatus, insect pest of cowpea during storage was highlighted by [27].

Concerning the contribution of mineral intakes, note that daily consumption of cowpea in Ivory Coast was 4.93 grams per person [40]. Regarding the average minerals contents found in cowpea seeds after 8 months of storage, the mineral contribution of the seeds in the triple bagging systems with biopesticide seems low, this being due to the low consumption of cowpea (4.93 g). However, the nutritional potential of cowpea seeds reveals an exceptional richness in mineral elements to such an extent that if the daily consumption of cowpea in Ivory Coast increased to 100 g following a change of diet, the mineral contents of cowpea seeds after 8 months of storage in the triple bagging systems with biopesticide would cover a satisfactory level of requirements for all minerals. The total intakes of Fe and Zn from seeds will contribute 80% and 50% respectively of the estimated daily recommendations.

Knowledge about mineral intakes of cowpea seeds could help the nutritionists to prepare dietary advice and preventative diets to reduce malnutrition. Thus, greater awareness of cowpea consumption should be conducted in rural areas where micronutrient deficiencies have become a public health problem for vulnerable populations.

5. CONCLUSION

The study confirmed the importance of setting up adequate systems (triple bagging with or without biopesticide) to preserve mineral quality of cowpea seeds. The triple bagging technique has shown its advantages in extending the shelf life of cowpea seeds for 6 months because at 7 months the contents for all minerals had dropped considerably. However, use of Lippia multiflora leaves made it possible to maintain the mineral composition of cowpea during the 8 months of storage. In addition, cowpea seeds from this technology (triple bagging associated with Lippia multiflora leaves) had considerable mineral quality for the recommended daily intakes. Therefore, the technology developed in this study could be an alternative to the use of synthetic pesticides in the protection of cereals and pulses. It is inexpensive, easy to perform and protects the environment and human health. This study must be deepened in order to preserve the vitamin quality of cowpea at the end of storage.

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