International Journal of Nutrition and Food Sciences
2023; 12(6): 173-179
http://www.sciencepublishinggroup.com/j/ijnfs
doi: 10.11648/j.ijnfs.20231206.12
ISSN: 2327-2694 (Print); ISSN: 2327-2716 (Online)

Study of the Conservation of the Anti-Oxidant Quality of Cowpea Seeds (*Vigna unguiculata* L. Walp) by a Triple Bagging System and *Lippia multiflora* Leaves

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To cite this article:
Fofana Ibrahim, Chatigre Kouamé Olivier, Ouattara Gninfanni Silvère, Biego Godi Henri Marius. (2023). Study of the Conservation of the Anti-Oxidant Quality of Cowpea Seeds (*Vigna unguiculata* L. Walp) by a Triple Bagging System and *Lippia multiflora* Leaves.*International Journal of Nutrition and Food Sciences*, *12*(6), 173-179. https://doi.org/10.11648/j.ijnfs.20231206.12

Received: October 15, 2023; **Accepted**: November 16, 2023; **Published**: November 29, 2023

Abstract: Cowpea seedsare a food legume rich in natural antioxidants. However, in Côte d'Ivoire, inefficient and sometimes unhealthy storage and preservation methods stand in the way of its production and food use. In the present work, triple-bottom bags combined or not with *Lippia multiflora* leaves have been proposed as a substitute for synthetic storage products. To evaluate this system, the phenolic compounds and antioxidant micronutrients of cowpea seeds were monitored over an 8-month period using a central composite design (CCD). Results for antioxidant quality parameters show a significant influence ($P < 0.05$) between treatment type and shelf life. After 8 months of storage, the rates of loss of total phenols, flavonoids, iron, zinc, manganese, α-tocopherol and percentage antioxidant activity of cowpea seeds recorded in triple-bottom bags combined with biopesticide were: 2-12%, 4-9%, 4-18%, 7-20%, 10-15%, 10% and 2-18% respectively. In the triple bagging system without biopesticide (H0), the values recorded after 4.5 months are respectively around 24%, 19%, 29%, 35%, 26%, 20% and 24% for total phenols, flavonoids, iron, zinc, manganese, α-tocopherol and antioxidant activity. Whereas in the control polypropylene bag (TST), those obtained after 4.5 months are respectively: 36%, 67%, 53%, 57%, 52%, 50% and 63%. Overall, the results obtained indicated a stable antioxidant quality of cowpeas during the first 5 months in the triple bagging system without biopesticide and during 8 months in the triple bagging systems combined with *Lippia multiflora* leaves. However, the minimum proportion of 2.5% *Lippia multiflora* leaves is desirable for maintaining the antioxidant quality parameters of preserved cowpea seeds.

Keywords: Cowpea Conservation, Ivory Coast, Biopesticide, Triple Bagging, Antioxidant Quality

1. Introduction

Cowpea (*Vigna unguiculata*) seeds, main source of plant protein, are also a potential source of essential nutrients (micro and phytonutrients) such as vitamins (α-tocopherol), minerals (iron, zinc, manganese) and phytochemical compounds that include phenolic compounds (total phenols, flavonoids) [1-4]. These nutrients are well known for their antioxidant actions and have been shown to have the capacity to prevent degenerative diseases in which reactive oxygen species are involved [5]. In developing countries, notably Côte d'Ivoire, cowpea seeds are increasingly used in feeding programs for vulnerable populations living in rural areas [6-8]. In these parts of the world, so-called civilization diseases remain a concern for public authorities. Rational consumption of cowpeas would therefore help prevent and treat these pathologies, which could affect vulnerable populations. Indeed, the consumption of cowpea seeds (rich in natural antioxidants) has been linked to the risk of reducing diseases caused by free radicals [9]. Unfortunately, the lack of good post-harvest storage and/or preservation practices does not guarantee the quality of cowpea seed nutrients, and militates against their use as food in rural areas. However, in recent decades, the use of triple-bottom systems (consisting of a double layer of high-density polyethylene placed inside a woven polypropylene bag) combined with aromatic plants leaves (biopesticides) has proved effective in preserving the health and nutritional qualities of cereals and legumes in rural Côte d'Ivoire [10-13].

However, little is known about the evolution of the antioxidant characteristics of stored cowpea seeds. Further studies on the antioxidant characteristics of stored cowpea seeds could contribute to the improvement and use of these preservation technologies. It is in this context that the present study, which aims to determine influence of triple bagging systems associated or not with *Lippia multiflora* leaves (biopesticide) on the antioxidant quality of cowpea seeds, was initiated.

2. Materials and Methods

Plant Material

Cowpea seeds: The cowpea seeds used were supplied in a mature, dry state by growers in the Loh-Djiboua region (5° 50′ North 5° 22′ West) just after harvesting. They belong to a local variety and have a white morphotype. These seeds are well known to growers and remain the most popular with consumers. However, they are the most susceptible to bruchid attack.

Lippia multiflora leaves: Fresh green leaves of *Lippia multiflora* (Verbenaceae) were harvested in the Gbéké region (7° 41′ North 5° 01′ West) and dried in the laboratory in the shade for 7 days. The dried leaves were ground using a microgrinder (CULATTI) fitted with a 10 µm mesh sieve.

Technical Equipment

The storage bags used consisted of polypropylene bags and triple bagging systems, all obtained in commune of Adjamé in Abidjan. The triple bagging systems obtained from suppliers consisted to 2 independent high-density polyethylene inner layers 80 mm thick. These 2 inner layers, with low air permeability, are fitted one inside the other, and placed in a woven polypropylene bag.

These bags have a storage capacity of 120 kg. The triple bagging technology used in this study was inspired by the PICS model and based on traditional methods.

Products and Reagents Used

All solvents and chemical reagents used in this study were of analytical grade. Methanol was supplied by Carlo-Erba (Spain). Gallic acid, aluminum chloride and 2,2diphenylpicrylhydrazyl (DPPH) were supplied by MERCK (Germany). Folin-Ciocalteu reagent and quercetin were supplied by Sigma-Aldrich (Germany). Vitamin standards were supplied by FlukaChimie (Switzerland) and Sigma-Aldrich (Germany).

Sampling

Immediately after harvesting and hulling the seeds, sampling was carried out at the major suppliers in the above-mentioned locality. A total of 306 kg of cowpea seeds were obtained from these suppliers. The untreated cowpea seeds were carefully placed in woven polypropylene bags and transported to the laboratory. Once at the laboratory, the seeds were sorted to separate them from harvest residues, and then a pool was made by mixing the different batches of samples. Out of all the samples, five (5) kg of cowpea seeds were taken for initial analysis prior to storage.

Cowpea Seed Preservation Method

The cowpea bagging preservation methodology was based on mixing a proportion of crushed dried leaves with a defined quantity of cowpea seeds as proposed by Konan *et al*. [14] modified. The experiment lasted 8 months and the whole set was packaged in triple-bottom systems and woven polypropylene bags. In total, one control batch and 5 experimental batches were formed as follows:

1) control batch: 50 kg cowpea packed in a biopesticide-free polypropylene woven bag (TST);
2) 1st experimental batch: 50 kg of cowpeas packed in a biopesticide-free triple bagging system (H0);
3) 2nd experimental batch: 50 kg cowpea packed in a triple bagging system containing 0.35 kg *Lippia multiflora* leaves, i.e. 0.7% biopesticide (H1);
4) 3rd experimental batch: 50 kg of cowpeas packed in a triple bagging system containing 1.25 kg of *Lippia multiflora* leaves, i.e. 2.5% biopesticide (H2);
5) 4th experimental batch: 50 kg of cowpeas packed in a triple bagging system containing 2.15 kg of *Lippia multiflora* leaves, i.e. 4.3% biopesticide (H3) and
6) 5th experimental batch: 50 kg of cowpea packed in a triple bagging system containing 2.5 kg of *Lippia multiflora* leaves, i.e. 5% biopesticide (H4).

The bags were filled alternately with cowpea seeds and *Lippia multiflora* leaves in stratum form.

Determining the antioxidant properties of stored cowpeas

The antioxidant property parameters measured during cowpea storage are phenolic compounds (total phenols, flavonoids and antioxidant activity) and antioxidant micronutrients (iron, zinc, manganese and vitamin E).

Extraction and determination of phenolic compounds

Phenolic compounds were extracted with methanol using the method of Singleton *et al*. [15]. To this end, one (1) g of cowpea powder was homogenized in 10 mL of methanol (70%, v/v). The resulting mixture was centrifuged at 1000 rpm for 10 min. The pellet was recovered in 10 mL methanol (70%, v/v) and centrifuged again. The supernatants were collected in a 50 mL flask and made up to the mark with distilled water. The resulting solution was called methanolic extract. Total phenols were determined according to the method of Singleton *et al*. [15], using the Folin-Ciocalteu reagent. The method used to determine flavonoid content was that of Meda*et al*. [16]. The principle of this method is based on the fact that, in the presence of potassium acetate, flavonoids react with aluminum chloride to give a yellow complex whose intensity is proportional to the quantity of flavonoids present in the medium. Antioxidant activity was determined using the method of Choi *et al*. [17].

Determination of antioxidant micronutrients (essential minerals and vitamin E)

Trace element levels (Fe, Zn and Mn) were determined using a scanning electron microscope (SEM) coupled to an energy dispersive spectrophotometer (EDS) according to the method described by AOAC [18]. Concentrations of α-tocopherol were determined using a high-performance liquid chromatography system (HPLC, Water Alliance brand) in accordance with standard 985.20 [18]. The system included a Waters pump, an automatic injector, a UV/PDA detector and a Servotrace recorder.

Statistical analysis

All tests relating to antioxidant property parameters were carried out in triplicate, and the numerical values obtained were expressed as the arithmetic mean affected by the corresponding statistical standard deviation.

Analyses of variance (repeated measures ANOVA and ANOVA 1) were performed using SPSS Statistical (version 22.0) and STATISTICA (version 7.1) software. The analysis of variance (repeated-measures ANOVA) with two classification criteria (type of treatment and duration of storage) was first performed on all results obtained during the first four and a half months of storage. It was then completed by a one-factor analysis of variance (type of treatment) for the remainder of the storage period (7 and 8 months), in order to determine the existence of statistically significant differences between the calculated averages. For these two types of statistical treatment, significant differences were highlighted using Tukey's test at the α significance level of 5%.

3. Results

Changes in phenolic compounds in stored cowpeas

The phenolic parameters assessed during cowpea preservation in this study concern total phenols, flavonoids and antioxidant activity. Statistical analysis of the data indicates a significant influence at the 5% threshold according to treatments over time (Tables 1 and 2). Total phenol, flavonoid and antioxidant activity levels dropped significantly ($P<0.05$) during storage.

Total phenols recorded at the start of storage with a mean value of 162.60 ± 0.58 mg/100g DM, decreased significantly ($P<0.05$) in the polypropylene control batch to a value of 103.73 ± 0.64 mg/100g DM after 4.5 months (destocking period). In the biopesticide-free triple bagging system, the total phenol content was estimated at 88.60 ± 6.23 mg/100g DM after 8 months' storage. In the other batches, i.e. the triple bagging systems with different proportions of biopesticides, total phenol content after 8 months' storage averaged 142.27 ± 0.00 mg/100g DM; 154.17 ± 7.31 mg/100g DM; 155.70 ± 4.27 mg/100g DM and 159.00 ± 1.00 mg/100g DM for H1, H2, H3 and H4 respectively. Thus, after 8 months of storage, statistical analysis at the 5% threshold indicates that there is a significant difference between the total phenol content of seeds stored in the triple bagging system without biopesticides and that of seeds stored in the triple bagging systems with different proportions of biopesticides, and between the total phenol content of seeds preserved with 0.7% of biopesticide and that of seeds with the other proportions (2.5%, 4.3% and 5%) of biopesticides (Table 2). Similarly, with a mean value of 111.57 ± 4.84 mg/100g DM at the start of storage (month 0), flavonoid levels dropped significantly ($P < 0.05$) during storage. The lowest values were recorded after 4.5 months' storage in the polypropylene control batch (36.57 ± 1.33mg/100g DM) and after 8 months' storage in the triple bagging system without biopesticides (87.05 ± 3.06 mg/100g DM). In the triple bagging systems with different proportions of biopesticide, the average flavonoid content recorded remains around 105.23 ± 1.29 mg/100g DM after 8 months' storage. Statistical analysis at the 5% threshold revealed a significant difference between the flavonoid content of seeds stored in the triple bagging system without biopesticides and those stored in the triple bagging systems with biopesticides (Table 2).

Concerning antioxidant activity, cowpea storage also revealed a significant ($P < 0.05$) decrease over time according to the type of treatment. Indeed, the antioxidant activity of cowpea seeds at the start of storage (95.02 ± 0.52% DM) dropped significantly to 35.30 ± 0.79% DM in the polypropylene bag (TST) after 4.5 months of storage and to 52.43 ± 0.63% DM in the triple bagging system without biopesticide (H0) after 8 months of storage. Antioxidant activity after 8 months' storage of cowpea seeds in the presence of different proportions of biopesticide was 78.13 ± 1.24% DM; 91.13 ± 0.32% DM; 92.60 ± 0.56% DM and 93.63 ± 0.72% DM for H1, H2, H3 and H4 respectively (Table 2).

Changes in antioxidant micronutrients in stored cowpeas

Statistical test data reveal highly significant variations ($P < 0.001$) in trace element and tocopherol content as a function of duration and type of treatment (triple bagging and/or biopesticide). In addition, the interaction between these two variables had a significant effect.

During the 8 months of storage, iron (Fe), zinc (Zn), manganese (Mn) and α-tocopherol were the 4 antioxidant micronutrients identified in cowpea seeds. The initial Mn content determined in the seeds was 36.03 ± 0.12 mg/kg. This content dropped significantly ($P < 0.05$) during the first 4.5 months of storage in the polypropylene control, to a value of 17.44 ± 1.09 mg/kg (Table 3). After 8 months' storage, a similar drop in Mn levels was observed in the triple bagging without biopesticide (19.17 ± 0.95 mg/kg) (Table 4). However, after 8 months of storage, the 1-factor ANOVA showed no significant difference ($P > 0.05$) between samples stored in triple bagging systems with different proportions of biopesticides in terms of levels of the above-mentioned mineral. The mean value recorded for manganese was 31.40

± 1.06 mg/kg.

Fe and Zn levels in the polypropylene control batch fell respectively 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 3). Decreases were also recorded in the triple bagging system without biopesticide (H0) which, after 8 months of storage, recorded contents of 77.87 ± 1.43 mg/kg for Fe and 29.80 ± 1.05 mg/kg for Zn (Table 4). Fe and Zn levels after 8 months' storage varied according to the type of treatment (proportion of biopesticide) in triple bagging systems combined with biopesticide ($P < 0.05$). However, these levels were still significantly higher than in 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 in H1, H2, H3 and H4 respectively (Table 4). Storage also revealed that α-tocopherol levels evolve differently in cowpea seeds over the storage period, which lasts 4.5 months for the polypropylene control bag (TST); 8 months for the triple bagging system without biopesticide (H0) and the 4 triple bagging systems with different proportions of biopesticide (H1: 0.7%; H2: 2.5%; H3: 4.3% and H4: 5%). With an average value of 0.10 ±0.01µg/100g before storage, α-tocopherol content decreased significantly ($P < 0.05$) over time. The lowest levels were obtained after 4.5 months' storage in the polypropylene control bag (0.05±0.01µg/100g) and 8 months' storage in the triple bagging system without biopesticide (0.05±0.01µg/100g). However, in the triple bagging systems in the presence of biopesticide, α-tocopherol levels remained constant and no significant difference ($P > 0.05$) was observed over the 8 months of storage, whatever the biopesticide concentration, with an average value of 0.09±0.01µg/100g (Table 4).

Table 1. *Changes in phenolic compounds in cowpea seeds preserved by different treatments for 4.5 months.*

Parameters	Shelf life (months)	Treatments					
		TST	H0	H1	H2	H3	H4
Total phenols (mg/100g)	0	162.60 ± 0.58^{aA}	162.60 ± 0.58^{aA}	162.60 ± 0.58^{aA}	162.60 ± 0.58^{aA}	162.60 ± 0.58^{aA}	162.60 ± 0.58^{aA}
	1	152.43 ± 2.56^{bB}	153.43 ± 0.59^{bB}	160.43± 1.53^{abA}	161.08± 1.15^{aA}	161.53 ± 1.16^{aA}	162.08 ± 2.56^{aA}
	2	134.63 ± 0.93^{cC}	143.53 ± 1.15^{bB}	158.70± 1.25^{abA}	160.03± 4.95^{abA}	160.10±3.05^{abA}	161.33 ± 0.55^{aA}
	4.5	103.73 ± 0.64^{dD}	122.93 ± 1.79^{cC}	154.00 ± 2.65^{bB}	158.17±6.90^{abAB}	158.43±2.37^{abAB}	160.90 ± 1.13^{aA}
Flavonoids (mg/100g)	0	111.57 ± 4.84^{aA}	111.57 ± 4.84^{aA}	111.57 ± 4.84^{aA}	111.57 ± 4.84^{aA}	111.57 ± 4.84^{aA}	111.57 ± 4.84^{aA}
	1	69.06 ± 0.46^{bC}	95.65 ± 5.16^{bB}	108.54±1.53^{abA}	109.07 ±1.10^{abA}	109.93 ± 0.26^{aA}	110.70 ± 1.22^{aA}
	2	49.60 ± 2.19^{cC}	94.90 ± 0.62^{bB}	106.63±1.10^{abAB}	107.33±1.20^{abAB}	109.47±2.05^{aA}	110.27 ± 3.05^{aA}
	4.5	36.57 ± 1.33^{dC}	90.07 ± 2.90^{cB}	105.40±1.37^{abAB}	106.83 ±1.25^{abA}	107.70 ±0.56^{abA}	108.30±1.44^{aA}
Antioxidant activity (%)	0	95.02 ± 0.52^{aA}	95.02 ± 0.52^{aA}	95.02 ± 0.52^{aA}	95.02 ± 0.52^{aA}	95.02 ± 0.52^{aA}	95.02 ± 0.52^{aA}
	1	85.21 ± 0.37^{bB}	94.00 ± 0.20^{aA}	94.30 ± 1.14^{aA}	94.47 ± 1.08^{aA}	94.71 ± 0.30^{aA}	95.00 ± 1.00^{aA}
	2	70.27 ± 0.67^{cC}	86.23 ± 1.36^{bB}	93.70 ± 0.36^{aA}	93.90 ± 0.72^{aA}	94.00 ± 0.35^{aA}	94.90 ± 0.10^{aA}
	4.5	35.30 ± 0.79^{dD}	72.23 ± 2.20^{cC}	90.03 ± 0.38^{bB}	93.03 ± 0.42^{abA}	93.70 ± 0.36^{aA}	94.73 ± 1.39^{aA}

Means (± standard deviation, n = 3) with different upper/lower case letters in the same row/column are different at the 5% threshold according to Tukey's test. With TST = Polypropylene bag control; H0 = Triple bagging without biopesticide; H1 = Triple bagging with 0.7% biopesticide (p / p); H2 = Triple bagging with 2.5% biopesticide (p / p); H3 = Triple bagging with 4.3% biopesticide (p / p); H4 = Triple bagging with 5.0% biopesticide (p / p). p / p: weight by weight.

Table 2. *Changes in phenolic compounds in cowpea seeds stored for 7 and 8 months according to the different treatments.*

Parameters	Shelf life (months)	Treatments				
		H0	H1	H2	H3	H4
Total phenols (mg/100g)	7	114.83 ± 5.46^{c}	148.57 ± 1.23^{b}	155.67 ± 4.70^{ab}	155.90 ± 3.08^{ab}	160.00 ± 0.40^{a}
	8	88.60 ± 6.23^{c}	142.27 ± 0.00^{b}	154.17 ± 7.31^{ab}	155.70 ± 4.27^{a}	159.00 ± 1.00^{a}
Flavonoids (mg/100g)	7	88.78 ± 4.24^{b}	103.90 ± 4.19^{a}	105.43 ± 1.76^{a}	106.93 ± 0.42^{a}	107.13 ± 0.35^{a}
	8	87.05 ± 3.06^{b}	101.77 ± 1.50^{a}	105.23 ± 1.29^{a}	106.77 ± 0.49^{a}	107.00 ± 0.70^{a}
Antioxidant activity (%)	7	55.73 ± 1.27^{c}	81.43 ± 1.03^{b}	92.30 ± 2.15^{a}	93.10 ± 0.88^{a}	94.07 ± 0.85^{a}
	8	52.43 ± 0.63^{d}	78.13 ± 1.24^{c}	91.13 ± 0.32^{b}	92.60 ± 0.56^{ab}	93.63 ± 0.72^{a}

Means ± standard deviation, n = 3

Means affected by the common letter on the same line are not significantly different from each other at the 5% threshold according to Tukey's test. With H0 = Triple bagging without biopesticide; H1 = Triple bagging with 0.7% biopesticide (p / p); H2 = Triple bagging with 2.5% biopesticide (p / p); H3 = Triple bagging with 4.3% biopesticide (p / p); H4 = Triple bagging with 5.0% biopesticide (p / p). p / p: weight per weight.

Table 3. *Changes in antioxidant micronutrient levels in cowpea seeds preserved by different treatments for 4.5 months.*

Parameters	Shelf life (months)	TST	H0	H1	H2	H3	H4
Iron (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}
Zinc (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}

Parameters	Shelf life (months)	TST	H0	H1	H2	H3	H4
	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}
Manganese (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}
α-Tocopherol (µg/100g)	0	0.10 ± 0.01^{aA}	0.10 ± 0.01^{aA}	0.10 ± 0.01^{aA}	0.10 ± 0.01^{aA}	0.10 ± 0.01^{aA}	0.10 ± 0.01^{aA}
	1	0.08 ± 0.03^{aAB}	0.09 ± 0.01^{aA}	0.09 ± 0.01^{aA}	0.10 ± 0.01^{aA}	0.10 ± 0.03^{aA}	0.10 ± 0.00^{aA}
	2	0.08 ± 0.03^{aAB}	0.08 ± 0.01^{aAB}	0.09 ± 0.01^{aA}	0.10 ± 0.01^{aA}	0.10 ± 0.01^{aA}	0.10 ± 0.01^{aA}
	4.5	0.05 ± 0.01^{bB}	0.08 ± 0.01^{aAB}	0.09 ± 0.01^{aA}	0.09 ± 0.01^{aA}	0.10 ± 0.02^{aA}	0.10 ± 0.01^{aA}

Means (± standard deviation, n = 3) with different upper/lower case letters in the same row/column are different at the 5% threshold according to Tukey's test. Where TST = Polypropylene bag control; H0 = Triple bagging without biopesticide; H1 = Triple bagging with 0.7% biopesticide (p / p); H2 = Triple bagging with 2.5% biopesticide (p / p); H3 = Triple bagging with 4.3% biopesticide (p / p); H4 = Triple bagging with 5.0% biopesticide (p / p). p / p: weight by weight.

Table 4. *Changes in antioxidant micronutrient content of cowpea seeds stored for 7 and 8 months according to the different treatments.*

Parameters	Shelf life (months)	H0	H1	H2	H3	H4
Iron (mg/kg)	7	81.60 ± 2.36^{c}	115.87 ± 3.14^{b}	124.77 ± 3.79^{ab}	128.27 ± 0.64^{a}	129.70 ± 2.71^{a}
	8	77.87 ± 1.43^{d}	110.70 ± 0.50^{c}	121.73 ± 2.10^{b}	127.50 ± 1.71^{a}	128.90 ± 1.01^{a}
Zinc (mg/kg)	7	31.07 ± 0.81^{c}	46.47 ± 0.68^{b}	51.07 ± 0.81^{a}	51.77 ± 0.96^{a}	52.60 ± 0.26^{a}
	8	29.80 ± 1.05^{d}	44.70 ± 0.53^{c}	49.43 ± 1.15^{b}	51.68 ± 0.46^{ab}	52.03 ± 0.81^{a}
Manganese (mg/kg)	7	25.03 ± 0.21^{c}	30.60 ± 0.95^{b}	31.30 ± 1.06^{ab}	31.80 ± 1.28^{ab}	33.60 ± 0.44^{a}
	8	19.17 ± 0.95^{b}	30.47 ± 0.81^{a}	31.17 ± 2.03^{a}	31.60 ± 1.25^{a}	32.37 ± 0.74^{a}
α-Tocopherol (µg/100g)	7	0.07 ± 0.01^{b}	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}	0.10 ± 0.01^{a}
	8	0.05 ± 0.02^{b}	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}	0.09 ± 0.00^{a}	0.09 ± 0.01^{a}

Means ± standard deviation, n = 3

Means affected by the common letter on the same line are not significantly different from each other at the 5% threshold according to Tukey's test. With H0 = Triple bagging without biopesticide; H1 = Triple bagging with 0.7% biopesticide (p / p); H2 = Triple bagging with 2.5% biopesticide (p / p); H3 = Triple bagging with 4.3% biopesticide (p / p); H4 = Triple bagging with 5.0% biopesticide (p / p). p / p: weight by weight.

4. Discussion

Increasingly, the exploration of new food sources with numerous beneficial effects on health potential in terms of functional or bioactive compounds (anti-oxidants) is at the heart of scientific food research. The results clearly show that cowpea seeds are rich in phenolic compounds and antioxidant micronutrients. Thus, the study of the behavior of these antioxidant parameters (total polyphenols, flavonoids, iron, zinc, manganese, α-tocopherol and antioxidant activity) during cowpea seed storage is of great importance insofar as the duration and method of storage are some of most important factors affecting the quantity and quality of chemical components of stored cowpea seeds. Overall, results obtained indicate that the combination of triple bags and *Lippia multiflora* leaves guarantees antioxidant quality of stored cowpea seeds. In fact, for these various antioxidant parameters, the highest levels were obtained in triple bagging systems combined with biopesticide, compared with single triple bagging and polypropylene bags, which recorded lowest values at the end of the experiment. Indeed, analysis of cowpea's antioxidant capacity indicates that all samples suffered a drop in total phenols, flavonoids, iron, zinc, manganese, α-tocopherol and percentage antioxidant activity. Sule *et al*. [4], also observed a drop in both total polyphenol, flavonoid and mineral contents, and in the percentage of antioxidant activity in cowpea seeds of different varieties. All the samples analyzed by Sule *et al* [4], showed variability in their content in terms of antioxidant properties. They suggest that polyphenoloxidase activity during seed storage is linked to the level of infestation. The work of Sule *et al* [4] and Mogbo *et al* [19] has shown that the significant drop in micronutrient content of cowpea seeds could be explained by the increased metabolic activity of insect populations. In Brazil, Ferreira *et al*. [20] also highlighted the decline in antioxidant properties of black bean seeds according to storage duration. Indeed, these authors have shown that the rates of decline in phenolic compound content and antioxidant capacity are a function of storage conditions [20]. However, low rates of decline for total phenols, flavonoids iron, zinc, manganese, α-tocopherol and antioxidant activity were observed in cowpea seeds contained in triple bagging systems at 4.5 months of storage, compared with cowpeas contained in the control batch (polypropylene bag), where the contents of total polyphenols, flavonoids iron, zinc, manganese, α-tocopherol and the percentage of antioxidant activity remained very low. After the first 5 months of storage, the levels of all these antioxidant parameters in the triple bagging system batch without biopesticides dropped, reaching low values after 8 months of storage. The combined effect of triple bagging and biopesticide maintained the levels of total phenols, flavonoids, iron, zinc, manganese, α-tocopherol and percentage antioxidant activity at high levels for up to 8 months of storage in the other experimental batches. The conservation of these antioxidant parameters, marked by low rates of decline, observed in batches in the triple bagging system during the first 5 months of storage may be explained by a decrease in oxygen content during storage. These observations could be similar to data on controlled atmospheres in cowpea pest control in hermetically sealed storage bags [21]. Indeed, low oxygen levels leading to high carbon dioxide (CO_2) levels after a certain period of time would inhibit the development of insects and molds largely responsible for the alteration of antioxidants in stored grains. The low rates of decline in antioxidant properties recorded after 8 months' storage could also be attributed to the insecticidal and/or insect repellent effect of *Lippia multiflora* leaves due to the release of essential substances (bioactive molecules). Our investigations are similar to those obtained by Konan [12] on cowpea seeds in Côte d'Ivoire. Indeed, the author demonstrated the efficacy of dried *Lippia multiflora* leaves against the development of pests responsible for seed spoilage. The results obtained are also in line with those of Akami *et al*. [22], who showed that essential oils of the *Lippia* genus would considerably reduce the development of *Callosobruchus maculatus* responsible for the alteration of the nutritional quality of cowpeas during storage.Indeed, the bioactive molecules of *Lippia multiflora* consist mainly of oxygenated monoterpenes such as linalool and 1,8 cineole [23]. These antimicrobial agents cause morphological and behavioral disturbances in pests.The prolonged effect of the bioactive molecules in *Lippia multiflora* leaves on cowpea at 8 months is due to the triple bagging system, which operates in a confined atmosphere, preventing any volatility of these molecules. Consequently, the combined effect of triple bagging and *Lippia multiflora* leaves resulted in acceptable antioxidant quality of cowpea after 8 months of storage.

5. Conclusion

The aim of this study was to preserve the antioxidant quality of cowpea seeds using a triple bagging system and *Lippia multiflora* leaves (biopesticide). To achieve this, the anti-oxidant quality parameters of cowpea seeds were assessed before and during preservation by the various processes over an 8-month period.

The results showed that preserved cowpeas evolve differently according to the treatment method. Storage time and treatment had a significant influence ($p < 0.05$) on storage quality. In fact, triple bagging systems preserved the antioxidant quality of cowpeas during the first 5 months of storage. However, from the seventh month of storage, only the triple bagging systems containing *Lippia multiflora* leaves as a biopesticide preserved the seeds better.

At the end of the 8 months of cowpea storage, low rates of decline in total polyphenol, flavonoid iron, zinc, manganese, α-tocopherol and antioxidant activity were recorded in the triple bagging systems with *Lippia multiflora* leaves added, compared with the triple bagging system without biopesticide and the polypropylene bag, which recorded the lowest values at the end of storage. Based on the study carried out, the recommended proportion of biopesticide is 2.5% of *Lippia multiflora* leaves (i.e. at least 1.25 kg of dried *Lippia multiflora* leaves mixed with 50 kg of cowpea seeds in triple bagging systems). This minimum level of biopesticide preserves all anti-oxidant parameters, in particular total phenol, flavonoid, iron, zinc, manganese and α-tocopherol contents, as well as the percentage of anti-oxidant activity in triple bagging systems.

Conflicts of Interest

The authors declare no conflicts of interest.

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