Evolution of Merchantability during Storage of Maize Triple Bagged Containing Biopesticides (Lippia multiflora and Hyptis suaveolens)

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Authors’ contributions
This work was carried out in collaboration among all authors. Author YVG designed the study, wrote the protocol, fitted the data and wrote the first draft of the manuscript. Author KKC checked the first draft of the manuscript and achieved the submitted manuscript. Authors NGL, AABA, AC and KKC performed the statistical analysis and assisted the experiments implementation. Author HMGB expertized the results interpretations. All authors managed the literature, read and approved the submitted manuscript.

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ABSTRACT

The aim of study was to evaluate merchantability quality of stored maize in triple bagging with biopesticides. Maize grains were collected in March 2016 in the north of Côte d’Ivoire. The fresh leaves of *Lippia multiflora* and *Hyptis suaveolens* were collected and dried in sunlight for 7 days in the center of Cote d'Ivoire. Triple bags were bought in Abidjan market. All this material was sent to the Laboratory of Biochemistry and Food Sciences, Félix Houphouët-Boigny University, Côte d'Ivoire, to perform the experiment. Ten treatments were obtained for the experimentation. The first treatment was conservation of 50 kg of maize grain in a polypropylene bag. The second treatment was conservation of 50 kg of maize grain in a PICS bag. The other eight treatments were carried out with PICS bags each containing 50 kg of maize grain and different proportions of chopped leaves *Lippia multiflora* and *Hyptis suaveolens*. A central composite design was used for sample constitution. Thus, a control group with polypropylene bags (TPP80), a control group in PICS bags without biopesticides (TPB0) and 8 experimental lots of triple bags noted TB1 containing 0.625 kg *L. multiflora* and 0.625 kg *H. suaveolens*, TB2 with 0.40 kg of *L. multiflora* and 1.60 kg of *H. suaveolens*, TB3 with 1.60 kg of *L. multiflora* and 0.40 kg of *H. suaveolens*, TB4 with 0.10 kg of *L. multiflora* and 0.40 kg of *H. suaveolens*, TB5 with 0.40 kg of *L. multiflora* and 0.10 kg of *H. suaveolens*, TB6 with 2.5 kg of *L. multiflora* and 2.5 kg of *H. suaveolens*, TB7 with 1.25 kg of *L. multiflora* and TB8 with 1.25 kg of *H. suaveolens* have been used. Changes in moisture, damages and weight losses were studied. The results show moisture levels (from 0.90±0.11% to 12.07± 0.06%), weight loss (from 0.49±0.02% to 2.54±0.07%) and damage (from 0.99±0.02% to 3.96± 0.01%), corn stored in triple bagged bags with different proportions of biopesticide were significantly lower than those recorded in the Polypropylene woven sample bag (TPPB0) and in the triple bagged control bag during the storage period. The results obtained indicate stability in the quality of maize stored for 18 months in triple bagged bags containing different proportions of leaves of *L. multiflora* and *H. suaveolens*. A proportion of 5% of the mixture of leaves of *L. multiflora* and *H. suaveolens* (2.5 kg of *L. multiflora* and 2.5 kg of *H. suaveolens*) in triple bagged bags is recommended for a better preservation of the merchantability of the stored maize grains kernels.

Storage of maize grains in PICS bags with the leaves of *L. multiflora* and *H. suaveolens* appears as a method of effective and inexpensive conservation to ensure the merchantability quality of maize.

**Keywords:** Stored maize; biopesticides; merchantability; triple bagging.

1. INTRODUCTION

Maize (*Zea mays*) is the most consumed cereal in Africa after rice. In addition, more than 300 million people in sub-Saharan Africa rely on maize as a source of food and subsistence [1], [2]. Of the twenty-two (22) countries in the world where maize is the highest percentage of caloric intake in the diet of populations, sixteen (16) are in Africa including Côte d’Ivoire [3]. In this country, maize (*Zea mays* L.) is distinguished by its very large extension of its cultivation area due to its great adaptability [4]. In 2014, its annual production was estimated at 680000 t [2]. It is consumed in various forms and is used in the preparation of several food recipes and in animal feed [5].

Today, maize is the subject of agricultural speculation and constitutes for some Ivorian populations, an important source of income [6]. While particular attention has been given to the factors of growth in production, namely the use of high-performance inputs (high-yielding maize varieties and the use of fertilizers), this has not been the case with storage and preservation. Thus, during the off-season and the lean periods, the food self-sufficiency of communities, both rural and urban, is becoming more and more difficult [7]. Maize suffers both qualitative and quantitative post-harvest losses due mainly to the action of insects, rodents and molds. The damage caused by the latter not only reduces the weight and the germinability of the grains but also degrades their nutritional, commercial and sanitary qualities [8]. They contribute to undermine the food supply of the population and reduce agricultural incomes. Quantitative losses approach 30 to 40% of production after six months of storage [9]. They are even higher in agroecological zones where the weather conditions are very favorable for the proliferation of insects.
It is estimated that 70 to 80% of cereal production, including maize produced in Côte d'Ivoire, is conserved at the village level in traditional storage structures, and it is precisely at this level that the highest losses are recorded high [10]. In the face of pest losses, contemporary control means consist of regular use of chemical pesticides [11]. Unfortunately, the use of the latter, in the fight against these pests, has a negative impact on the health of the consumer and the environment [12]. In Côte d'Ivoire, organochlorine chemical pesticides were found at concentrations ranging from 2 to 59.7 μg / kg in cocoa bean stocks and between 2 and 237 μg / kg in kola nut stocks [13,14]. It is important to face these problems, to find other alternative methods of fight accessible to the farmers, respectful of the environment and guaranteeing the health of the consumers. Numerous studies have shown the effectiveness of hermetic systems in the control of stock pests [15,16,17]. The most used and most practical technology in the peasant environment is triple bagging or PICS bag. This storage method was initiated by the Purdue American University in Niger for the storage of cowpea. In addition, other studies have shown the insecticidal or repulsive properties of certain aromatic plants (Neem, Lippia multiflora, Hyptis suaveolens) during post-harvest food storage [10,18]. In addition, studies [18] showed the synergistic effect of the triple bagging system and Lippia multiflora leaves on the quality of cowpea grains during storage. Thus, this study aims to evaluate the marketability of maize grains stored in a triple bagging system in the presence of leaves of L. multiflora and H. suaveolens.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was performed at Laboratory of Biochemistry and Food Sciences (LABSA) UFR Biosciences at the University Félix HOUPHOUET-BOIGNY. The different bags were kept in a laboratory storeroom to 27.78±0.19°C temperature and 75.0±0.99% relative humidity. Wooden pallets were arranged floored as support for triple bagging system.

2.2 Collection of Maize Grains and Biopesticides Plants used in the Study

Maize grains and leaves of L. multiflora and H. suaveolens were collected in March 2015 from producers of Gbékê region (7º50 North and 5º18 West in center of Côte d'Ivoire). Prior to the storage, maize were sun-dried for 2-3 days before being used for the experiment. While, the L. multiflora and H. suaveolens leaves were drying at an average temperature of 30°C for 6-7 days, and kept away from direct sun exposure. The dried leaves were chopped into fine particles before being used for the experiment.

2.3 Implementation of Experiment

2.3.1 Using the triple bagging

Storage bags used in our study, were made of polypropylene bags and polyethylene bags (Purdue Improved Cowpea Storage: PICS) developed by Purdue University for storing cowpeas from Niger. These bags, obtained from suppliers, are composed of a triple bagging system.

2.3.2 Treatments

The implementation of the study was conducted from March 2015 to September 2017. The storage method is based on the mixture of plants leaves. Method tested in this study, consisted in adding of biopesticides (0-5% w/w) in the polypropylene bags and the triple bagging system containing 50 kg maize grains and storing on pallets in warehouses for 18 months. The filling of the bags was performed by alternately as maize grains strata and biopesticides. The maize grains were stored as follows:

- 1 control batch of 50 kg of maize grain in polypropylene bag without biopesticide (TPB0);
- 1 control batch of 50 kg of maize grain in triple bagging system without biopesticide (TPPB0);
- 1 experimental batch of 50 kg of maize grain in triple bagging system with 2.5% of biopesticides (0.625 kg L. multiflora and 0.625 kg H. suaveolens) (TB1);
- 1 experimental batch of 50 kg of maize grain in triple bagging system with 3.99% de biopesticides (0.40 kg L. multiflora and 1.60 kg H. suaveolens) (TB2);
- 1 experimental batch of 50 kg of maize grain in triple bagging system with 3.99% de biopesticides (1.60 kg L. multiflora and 0.40 kg H. suaveolens) (TB3)
2.4 Sampling

The sampling was performed at the beginning of the storage (0 month), then 5, 10, 15 and 18 months later, in triplicate. Thus, 2 kg of maize samples from each bag was gathered through the top, the center and the bottom opening sides.

2.4.1 Determination of moisture content

The moisture content was valued according to the method described by AOAC [19]. A maize sample of 5 g was dried at 105°C into an oven till constant weight. The result was expressed from the equation 1 below:

\[
\text{Moisture content (\%) } = 100 - \frac{\text{WI} \times 100}{\text{Ws}}
\] (1)

With WI, weight lost from samples after drying; Ws, weight of raw samples.

2.4.2 Assessment of damage and weight loss

To assess the damage caused by insects during storage, samples of 1 kg (approximately 3500 maize kernels) were taken. After sifting and removal of the foreign matters, the grains were weighed and sorted to separate attacked and damaged grains from healthy grains. Then, the two fractions were weighed and counted separately. The percent grain damage was estimated using the method of counting and weighing of Harris and Lindblad [20], [21]. Assays were performed in duplicate. Thus, the rate of infection is the ratio of grains having at least one hole in the total number of grains. The estimate of the damage (D) and weight loss (W) is given by the formulas:

\[
D (\%) = \frac{\text{NGA}}{\text{NTG}} \times 100
\]

\[
W (\%) = \frac{\left[\frac{(\text{NGA} \times \text{WHG}) - (\text{NHG} \times \text{WAG})}{\text{WHG} \times \text{NTG}}\right]}{100}
\]

NGA = Number of grains attacked; NTG = Total Number of grains

W (%) = \left[\frac{(\text{NGA} \times \text{WHG}) - (\text{NHG} \times \text{WAG})}{\text{WHG} \times \text{NTG}}\right] / 100

NGA = Number of grains attacked; NHG = Number of healthy grains; NTG = Total Number of grains; WAG = Weight of attacked grain; WHG = Weight of healthy grains.

2.4.3 Statistical analysis

All analyses were performed in triplicate and the full data were statistically treated using SPSS software (version 20.0). It consisted in Analysis of Variance at repeated measures. Means derived from parameters were compared with the Tukey High Significant Difference test at 5% significance level. Correlations between parameters were also assessed according to the Pearson index. Then, Multivariate Analyses through Principal Components Analysis (PCA) and Ascending Hierarchical Clusters analysis (AHC) were performed using STATISTICA software (version 7.1).

3. RESULTS

Statistical analysis of the data indicates a significant influence at the 5% level of both duration and type of packaging on the assessed market quality parameters (Table 1).

3.1 Evolution of Moisture during Storage

Fig. 1 shows the evolution of the moisture content of maize grains stored in different batches. With an average of 9.02±0.01% initially (0 months), the moisture content increased significantly (\( P = .001 \)) during the storage period (Table 2). For the polypropylene bag control batch (TPPB0), the moisture content increases sharply to 16.99±0.02% after 18 months of storage. As for the control group triple bagging system (TPB0), after 18 months of storage, the moisture content is 13.16±0.10%. With regard to the lots stored in bags in triple bagging system with different proportions of biopesticide (TB1, TB2, TB3, TB4, TB5, TB6, TB7 and TB8), the moisture contents are similar after 18 months of storage and have an average value of 12.20 ± 0.05%.
3.2 Evolution of Weight Losses during Storage

At 0 months, the losses are 0.49±0.02% for all treatments (Fig. 2). In the polypropylene control batch (TPPB0), this value increases rapidly and significantly, reaching a rate of 49.53±1.04% at 18 months. With regard to the other batches, that is to say the batches in triple bagging system with different proportions of biopesticide, the weight losses remained low, ranging from 0.49±0.02% to an average value 3.40±0.13% during the 18 months of storage (Table 2).

3.3 Evolution of Damage during Storage

Fig. 3 shows the evolution of insect damage in stored corn kernels. Before storage (0 months), the damage recorded is 0.99±0.02%. This damage progressed rapidly in the polypropylene control batch to reach the value of 26.15±0.01% in 5 months and thereafter a value of 54.30±0.51% after 18 months of storage. With regard to the control group in triple bagging system (without biopesticide), there was a significant increase in the damage from the 15th month, from 5.63±0.11 to 12.5±0.09% at the 18th month.

Table 1. Statistical data for moisture, weight losses and damages during maize storage

| Source of variation           | Parameters          | Losses  | Damages | Moisture |
|------------------------------|---------------------|---------|---------|----------|
| Treatments (T)               | df                  | 9       | 9       | 9        |
|                              | SS                  | 10004.51| 12470.67| 171.43   |
|                              | F-value             | 2025.18 | 3130.55 | 3590.54  |
|                              | P-value             | < .001  | < .001  | < .001   |
| Error Treatments             | df                  | 20      | 20      | 20       |
|                              | SS                  | 10.98   | 8.85    | 0.11     |
| Storage duration (months)    | df                  | 1.12    | 1.21    | 3.54     |
|                              | SS                  | 1420.04 | 1938.51 | 382.63   |
|                              | F-value             | 1534.67 | 2222.57 | 16985.88 |
|                              | P-value             | < .001  | < .001  | < .001   |
| Error Duration               | df                  | 22.43   | 24.12   | 70.88    |
|                              | SS                  | 18.51   | 17.44   | 0.45     |
| Treatments x Duration        | df                  | 10.09   | 10.86   | 31.90    |
|                              | SS                  | 5872.32 | 7167.71 | 70.78    |
|                              | F-value             | 705.15  | 913.11  | 349.12   |
|                              | P-value             | < .001  | < .001  | < .001   |

SS: Sum of Squares; F-VALUE: value of the statistical test; P-VALUE: probability value of the statistical test; df: degree of freedom
of storage. In biopesticide triple bagging lots, the damage varied slightly compared to control batches with values from 6.13±0.15 to 6.21±0.12 for batches TB1, TB4 and TB5, and from 3.96±0.09 to 4.11±0.06 for batches TB2, TB3, TB6, TB7 and TB8 after 18 months of storage (Table 2).

3.4 Distribution of Individuals in PCA Plan 1-2 and Hierarchical Ascending Classification

The principal components analysis was carried out using the F1 component which records an eigenvalue higher than 1, according to the Kaiser rule. However, the second component F2 (eigenvalue 0.21) is associated with the first component for the representation of the PCA. Fig. 4 shows the correlation circle between the F1-F2 components representing 99.94% of the total variability and the set of parameters of the marketability of the stored maize. The projection of the stored maize samples divides the individuals into 2 groups. Group 1 consists essentially of individuals from the control batch in polypropylene bag of 5 to 18 months of storage (denoted TPPB0). These individuals are superimposed on characters negatively correlated to factor F1, characterized by high values of moisture, weight loss and damage. The second group contains all the samples of the lots in triple bagging system with or biopesticides and the control batch in polypropylene bag with 1 month of storage. These are distinguished by low values in the different parameters.

![Fig. 2. Evolution of weight losses of stored grains maize during 18 months](image2)

![Fig. 3. Evolution of damages of stored grains maize during 18 months](image3)
### Table 2. Evolution of moisture content, weight losses and damages of maize stored during 18 months

| Parameters | Duration (months) | TPPB0 | TPB0 | TB1 | TB2 | TB3 | TB4 | TB5 | TB6 | TB7 | TB8 |
|------------|-------------------|-------|------|-----|-----|-----|-----|-----|-----|-----|-----|
| Moisture content (%) | 1 | 9.02 ± 0.01 \(a\) | 9.02 ± 0.01 \(a\) | 9.02 ± 0.01 \(a\) | 9.02 ± 0.01 \(a\) | 9.02 ± 0.01 \(a\) | 9.02 ± 0.01 \(a\) | 9.02 ± 0.01 \(a\) | 9.02 ± 0.01 \(a\) | 9.02 ± 0.01 \(a\) | 9.02 ± 0.01 \(a\) |
| Weight losses | 1 | 10.20 ± 0.10 \(b\) | 9.23 ± 0.06 \(ab\) | 9.10 ± 0.02 \(a\) | 9.09 ± 0.07 \(a\) | 9.07 ± 0.04 \(a\) | 9.17 ± 0.08 \(a\) | 9.12 ± 0.01 \(a\) | 9.08 ± 0.03 \(a\) | 9.12 ± 0.03 \(a\) | 9.09 ± 0.04 \(a\) |
| Damages | 1 | 14.05 ± 0.07 \(c\) | 11.37 ± 0.08 \(bc\) | 11.02 ± 0.13 \(ab\) | 10.96 ± 0.06 \(ab\) | 10.96 ± 0.13 \(ab\) | 11.08 ± 0.07 \(a\) | 10.96 ± 0.06 \(ab\) | 10.92 ± 0.06 \(ab\) | 10.98 ± 0.09 \(ab\) | 10.92 ± 0.07 \(ab\) |

The mean (± SD) with different lowercase / uppercase letters on the same line / in the same column are different test probability of 5%. TPPB0 = Control with polypropylene bag; TPB0 = Control with PICS bag (no biopesticide); TB1 = PICS bag with 2.5% of biopesticide (0.625kg L. multiflora and 0.625kg H. suaveolens) (w / w); TB2 = PICS bag with 3.99% biopesticide (0.40kg L. multiflora and 1.60 kg H. suaveolens) (w / w); TB3 = PICS bag with 3.99% of biopesticide (1.60 kg L. multiflora and 0.40 kg H. suaveolens) (w / w); TB4 = PICS bag with 1.01% of biopesticide (0.10 kg L. multiflora and 0.40 kg H. suaveolens); TB5 = PICS bag with 1.01% de biopesticide (0.40 kg L. multiflora and 0.10 kg H. suaveolens); TB6 = PICS bag with 5% de biopesticide (2.5 kg L. multiflora et de 2.5 kg H. suaveolens); TB7 = PICS bag with avec 2.5% de biopesticide (1.25kg L. multiflora); TB8 = PICS bag with 2.5% de biopesticide (1.25kg H. suaveolens)
Fig. 4. Correlation drawn between the F1-F2 factorial of the principal components analysis and the merchantability parameters (a) and the individuals (b) deriving from the maize samples studied

4. DISCUSSION

The use of natural substances to improve the efficiency of storage structures has been demonstrated in previous studies [10]. The leaves of Lippia multiflora and Hyptis suaveolens used for this study have a positive influence on the marketability of stored corn kernels. The low rates of weight loss and damage observed in triple bagging systems treated with biopesticides may be due to the presence of these leaves. These results are similar to the work of Gueye, et al. [9] in the Kédougou region of eastern Senegal, which showed the repellent effect of the dried leaves of Hyptis spicigera and Hyptis suaveolens against the insect pests of cereals Tribolium castaneum and Sitophilus zeamais in traditional granaries for a period 7 months. These results are also consistent with the work of Ukeh, et al. [22] conducted in Obudu, South-East Nigeria, which showed that 10% (w / w) powders and Aframomum melegueta and Zingiber officinale (Zingiberaceae) essential oils significantly reduce the progeny of Coleoptera Sitophilus zeamais type in traditional African granaries over a period of about 3 months. These results obtained in this study are in agreement with our results. These insecticidal properties are attributed to the presence of terpenes, such as linalool for L. multiflora and β-caryophyllene for H. suaveolens [23]. In addition, the optimization studies carried out by Biego and Chatigre [24] on the storage of corn, have also shown that the leaves of L. multiflora and H. suaveolens significantly improve the conservation of maize grains stored in polypropylene bags during 6 months.

The low levels of the marketability parameters obtained in triple bagging systems, during the 18 months of storage, could not be attributed solely to the activity of biopesticides. They can also be explained by the packaging of grains which is hermetic type. Indeed, the polyethylene plastic used constitutes an impermeable barrier against the ambient air. Insects requiring oxygen for their development are in a low-oxygen and high-carbon dioxide (CO2) environment that inhibits their development [15]. The studies by Baoua, et al. [16] confirm our results on the reduction of weight loss and damage through the use of triple bagging system. These authors showed that the triple bagging system would preserve the corn kernels against the maize insect pests Prostephanus truncatus Horn, Sitophilus zeamais Motschulsky and Rhyzopertha dominica (F.). Other authors have also shown the effectiveness of hermetic containers against P. truncatus in Mexico, resulting in 100% mortality of this pest after only a few days of storage [25].

These results obtained represent, for the producer, a significant reduction in insect damage of 78% and a weight gain of 83% after 18 months of storage for the lots in triple bagging
system compared to the control batch in polypropylene bag. The presence of biopesticide in triple bagging systems increases damage and weight loss reduction to 93% and 95%, respectively, after 18 months of storage. Indeed, according to Boone, et al. [26], maize is mainly produced by small producers who need the results of research to safeguard their production given low production yields. Maize storage in triple bagging provides many benefits to smallholder farmers. This is the availability of quality corn for human, animal and industrial food. The sale of stored maize could provide small producers with substantial income [26]. This will allow them to speculate on selling prices for better income. In sum, the triple bagging system combined with a minimum concentration of 5% of biopesticide (2.5 kg of *L. multiflora* and 2.5 kg of *H. suaveolens*) make it possible to preserve the commercial quality of corn stored for 18 months. Our method is an interesting alternative for maize storage, in perfect balance with the environment, the health of producers and consumers [18].

5. CONCLUSION
The assessment of merchantability during maize storage showed a progressive degradation of these parameters. However, the presence of the leaves of *L. multiflora* and *H. suaveolens* combined with the triple bagging system makes it possible to preserve the commercial quality of the corn (moisture, weight loss and damage) during 18 months of storage. This technique makes it possible to make the maize available during the seasons. It is easily applicable and contributes to the protection of the environment.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

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