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Changes in the Quality of Maize (*zea mays* l.) Post-Harvest Stored in Granaries with the Biopesticides from Rural Conditions in Côte d'Ivoire

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Abstract

The aim of this study was to determine the correlation between merchantable, nutritive and hygienic qualities of maize (*Zea mays* L.) cobs and grains stored in traditional and improved granaries using a combination of leaves derived from *Lippia multiflora* Moldenke and *Hyptis suaveolens* Poit. Benth. Thus nine parameters were studied and monitoring during 8 months of storage. Results showed that all the parameters were strongly linked and significantly ($P<0.05$) altered during the storage period of the study. The contents of water (0.83 to 0.91), moisture (9.14% to 12.78%), weight loss (0.02% to 34.32%), free fat acidity (2.00% to 5.88%), peroxide (2.10 meq O_2/kg to 6.10 meq O_2/kg), aflatoxin B_1 (0.28 µg/kg to 58.10 µg/kg) and ochratoxin A (0.32 µg/kg to 41.53 µg/kg) increase whereas a decrease in starch content (65.10% to 53.5%) and iodine value (121.40 g I_2/100 g to 94.82 g I_2/100 g) were recorded. For each stage, composition of maize cobs and grains did not differ whether they are treated in traditional or improved granaries with both plant materials. The results also indicate that storage maize at 6 months with the combination of two local plants would be more suitable. Thus, the use of leaves derived of *L. multiflora* and *H. suaveolens* can slow down the degradation of maize in storage.

Keywords: *maize stored, quality, biopesticides, traditional and improved granaries*

1. Introduction

Cereals, in general and maize, in particular constitute a solution to the high food demand throughout the world. Indeed, it is one of the staple foods in Africa, Asia and Latin America (Di Domenico *et al.*, 2015; Agbobli *et al.,* 2007). In southern Africa, for instance, maize has become the most important staple food and supplies more than 50% of the energy in local diets. Global statistics for cereal consumption calculated by the World Health Organization indicate average total cereal consumption in the African diet is 291.7 g/person/day, including an average maize consumption of 106.2 g/person/day (Cissé *et al.,* 2013). In Côte d'Ivoire, the mean daily consumption of maize grains is estimated at 28.4 g (Beugre *et al*., 2014). It allows diverse dishes such as porridge, couscous or dense paste (tô) eaten with sauce (Nguessan *et al*.,2014).

However, after harvest, inadequate infrastructure and lack of economic means usually involve in storage of maize crops by farmers, either shelled or unshelled using traditional structures and processing, such as living rooms, cribs, baskets, polypropylene bags, earthen ware and granaries (Niamketchi *et al*., 2015). Unfortunately, crops kept in these conditions and structures are generally subject to deterioration. The primary factors affecting the grains during their storage are the moisture, the temperature and the relative humidity of the environment. Other maize deterioration agents are rodents, insect pests and microorganisms. Both primary and secondary factors lead to chemical changes (nutritive and hygienic parameters), weight loss and finally to changes in the maize quality (Kent and Evers, 1993). These are so important damages that the farmers often dispose of significant proportion of their stored grains due to deterioration. In general, infestations start at fields and continue throughout the storage period (Johnson *et al*., 2012).

The full losses resulting with deterioration are about 25-30% of the stored food grains (Gueye *et al.,* 2011). Thus, proper conditions of maize storage could allow significant improvement in the national farmer's economy by controlling the losses (Chattha *et al.,* 2015).

In fact, the storage technologies have major roles upon the final quality of the resulted grains. Ensuring optimal efficiency of the storage technologies is highly crucial for the safety of stored grain and for the consumer's health. Common pests controlling system of stored products is with the application of synthetic contact insecticides (Nukenine *et al.,* 2013) despite many risks on the health of users and consumers as well as the environmental pollution (Regnault-Roger, 2008). Nevertheless, other methods of storage and conservation could be improved in order to find alternative in uses of synthetic pesticides for the post-harvest losses reduction.

The objective of the current research is to establish the most efficient, economically feasible and safe storage structure that would benefit to farmers. The study assesses effects of two local plants *Lippia multiflora* and *Hyptis suaveolens*, deriving with the quality of maize stored in traditional and improved clay granaries in rural conditions of Côte d'Ivoire.

2. Materiel and Methods

2.1 Experimental site

Experiments were carried out in the rural farming community of Djedou village in the department of Botro, Gbèkê region, in the center of Côte d'Ivoire (Figure 1). The village is located at 40 km from Bouaké, with points of 7°50' N and

5°18' W. This region has a humid tropical climate with annual rainfall ranging between 1000 and 1100 mm, mean temperatures of 21.4°C to 30.6°C and 75% to 80% of relative humidity (CNRA, 2014).

2.2 Collection of the maize used in the study

Maize grains and full maize cobs were bought in January 2014, approximately one month after harvest, from the young cooperative of the Djedou village. Prior to the storage, maize were sun-dried for 2 to 3 days before being used for the experiments.

2.3 Biopesticides collection and processing

Two plants species *Lippia multiflora* and *Hyptis suaveolens* have been selected for their biopesticides properties. Both plants are spontaneous perennial and fragrant shrubs growing from the central to the Northern parts of Côte d'Ivoire (Ekissi *et al.*, 2014; Tia, 2012). Leaves of *L. multiflora* and *H. suaveolens* were collected around Djedou village. After harvest, the leaves have been dried out of direct sunlight for 6-7 days.

2.4 Experiments implementation

2.4.1 Granaries main parameters

A cylindrical clay granary covered with a straw roof side was chosen for the experiment. Such convenience is commonly used by farmers to keep their cereal crops (maize, rice, millet, sorghum). The granaries are built by a specialist farmer after the main fieldwork. Such operation runs from 1 to 12 months. To relieve the difficulties encounted, traditional granaries are modified by replacing their cylindrical roof with a simple device in similar design. The straw roof has been substituted with a plastic for hermetical recovering of granaries. Besides, granaries are raised from the ground to prevent moisture and rodent attack. Such systems reveal general storage capacity of 9 m^3 to 12 m^3 (Photography 1).

2.4.2 Experimental design

The experiment was carried out using a completely randomned 3x4 factorial design with two forms of maize: cobs and grains. Factors were three types of granaries (control, traditional and improved) and four observation periods (0, 2, 6 and 8 months). The investigation runned from January to September 2014 and the young cooperative of Djedou village was associated. The maize grains storage granaries were built in Djedou village; and the maize cobs storage granaries were located at N'godrjenou camp, 4 km far from Djedou, to facilitate the surveillance and monitoring. Excepted for the control, granaries contained mixtures of chopped dried leaves of *L. multiflora* and *H. suaveolens* at 2.5% w/w of each plant. The required quantities of each plant material were intermittently sandwiched manually in granaries, after 120 kg of maize cobs or grains.

2.4.3 Sampling

The sampling was performed at the beginning of the storage (0 month), then 2, 6 and 8 months later, in triplicate. Thus, 4 kg maize samples from each granary were gathered through the top, the centre and the bottom opening side. Maize samples were then conveyed to laboratory for the physicochemical and mycotoxins parameters assessments.

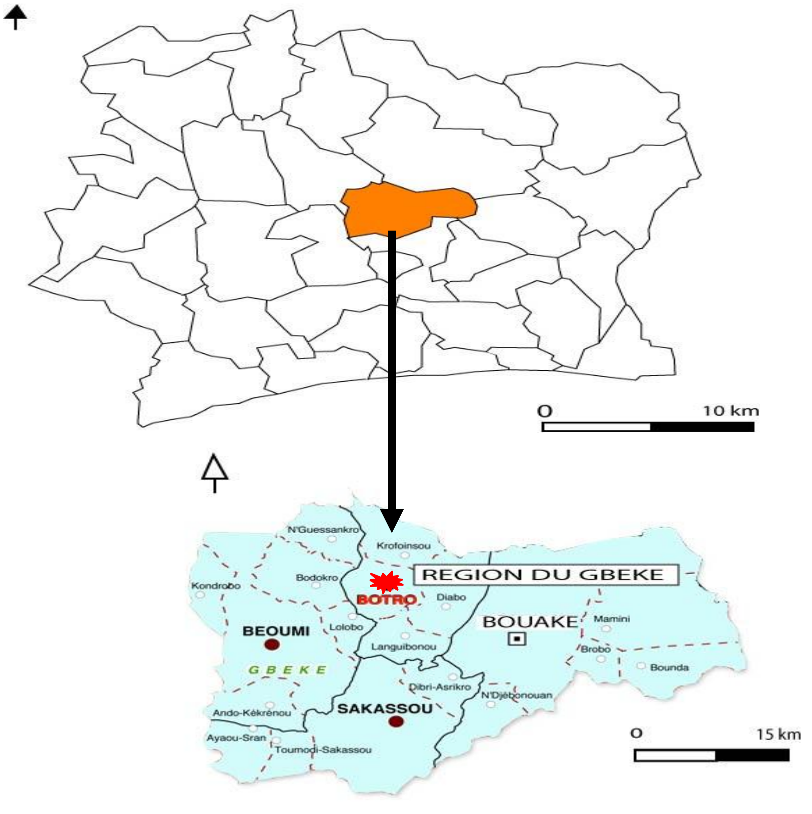
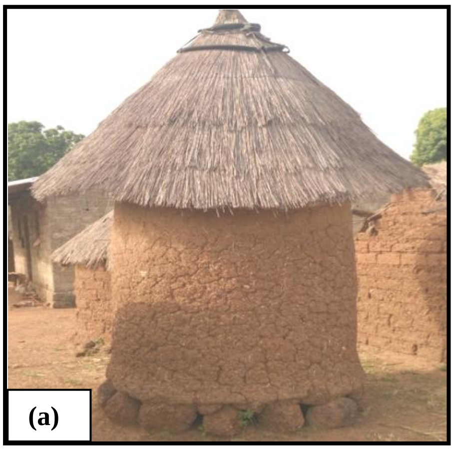


Figure 1: Distribution map of the study zone



(a) Control granary for maize storage;

(b) Traditional granary for maize storage;

(c) Improved granary for maize storage

Photography 1 : Different types of maize granaries storage using for experiments implementation

2.5 Analysis of quality of cobs and grains maize stored

2.5.1 Physicochemical analysis

Gravimetric method was used for moisture determination using an oven (MEMMERT, Germany) at 105°C with the official methods of AOAC, 2000. The water activity was ascertained using a hygrometer from HygroLab Rotronic according to the method of McCormick (1995). Thus, a sample of 5 g of maize grains was placed in 10 Aw containers void of any trace of water. After two minutes, value of water activity was directly carried out in the device. Starch content was determined using iodine method of Jarvis and Walker (1993). The determination of the rate of free fatty acids consisted first in the extraction of the maize fat to the soxlhet hexane during 8h (AOAC, 2000). Then, the fat acidity value was determined by titrating the diethyl ether / ethanolic solution of maize oil with an ethanolic solution of sodium hydroxide using phenolphthalein indicator (AOAC, 2000). Finally, the rate in free fatty acids (FFA) was expressed as the percentage of oleic acid per gram of maize fat content, calculated according to the following relationship:

$$FFA = \frac{Volume\ in\ mL\ of\ KOH \times N_{KOH} \times 282}{maize\ fat\ mass} \qquad \textbf{(1)}$$

Molar Mass of the oleic acid = 282 g/mol.

The peroxide value was determined by titrating chloroform/glacial acetic acid/potassium iodide solution of maize oil with an aqueous solution of sodium thiosulphate using starch as indicator (AOAC, 2000). The iodine value was determined by the Wijs' method (AOAC, 2000). The assessment of insects in the maize products was made by visual counting in 1 kg of maize sampled using the following formula (Harris and Linblad, 1978):

$$W (\%) = [[(NGA \times PGS) – (NHG \times WAG)] / (WHG \times NTG)] \times 100 \quad (2)$$

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.

2.5.2 Determination of aflatoxin B_1 and ochratoxin A

All reagents were of analytical grade. Acetonitrile, methanol and chloroform were purchased from Carlo Erba (Spain). The aflatoxin and ochratoxin standards were purchased from Sigma (Sigma, St Louis, MO, USA).

2.5.2.1 Extraction and purification of aflatoxin B_1

Aflatoxins B_1 was extracted and purified following official AOAC method (AOAC, 2005). In an erlenmeyer flask containing 25 g of ground maize, 100 mL of a methanol-water (v/v, 80:20) mixture were added. The solution was homogenized for 2 minutes and stored in darkness at room temperature for 12 hours. Then it was filtered with a Whatman N°4 filter paper and 50 mL of the filtrate were added to 40 mL of a phosphotungstic acid-zinc sulfate-water (5/15/980, w/w/v) mixture and kept at ambient temperature for 15 min. This second solution was filtered on Whatman N°4 filter paper in a flask. Aflatoxins were extracted from the second filtrate with 3 volumes of 10 mL of chloroform. The extracts were collected into a 50 mL flask and then evaporated with use of a rotative evaporator (Buchi Rotavapor R-215) at 40 °C. To the dry extract were added 0.4 mL of hydrochloric acid and 4.6 mL of bidistillated water. And the final solution was filtered through filter Rezist in a chromatographic tube and then passed through an immunoaffinity column (column RiDA aflatoxin, Biopharm, Germany).

2.5.2.2 Extraction and purification of OTA

The entire sample was crushed in a hammer mill to obtain a homogeneous fine grind. In a Nalgene jar containing 15 g of homogenate, 150 mL of aqueous methanol-bicarbonate 1% (m / v, 50:50) were added. The mixture was homogenized by Ultra-Turax for 3 minutes and the homogenate was centrifuged at 5000 rpm for 5 min at 4 ° C. The supernatant was filtered through filter paper into tubes of 25 mL. To 11 mL of filtrate were added 11 ml of saline phosphate buffered (PBS) at pH 7.3. Immunoaffinity columns brand Ochraprep and R-Biopharm were conditioned with 10 mL of PBS. Purification of 20 ml of the mixture was made on immunoaffinity columns and OTA extraction was performed with two volumes of 1.5 mL of PBS at a flow rate of 5 mL / minute. The resulting sample was packed in a chromatographic tube and the analysis of OTA was made by HPLC using the European community regulation (CE 401/2006).

2.5.2.3 AFB_1 and OTA quantification

A liquid chromatograph HPLC brand Shimadzu coupled to a fluorescence detector was used and the operating conditions are described in Table I.

Table I: Conditions of aflatoxin B1 and ochratoxin A analysis by HPLC

ITEM	AFLATOXIN B1	OCHRATOXIN A
Pre-column	Shim-pack GVP-ODS 10 x 4.6 mm	
Column	Shim-pack GVP-ODS, 250 mm x 4.6 mm	
Detector	Fluorescence, λ excitation : 365 nm,	Fluorescence, λ excitation: 330 nm,
	λ emission : 435 nm	λ emission: 460 nm
Mobile phase	Methanol/Water / Acetonitrile (60/20/20)	Acetonitrile/Water/Acetic acid (99/99/2)
Inject volume	20 µL	100 µL
Flow rate	1 mL/minute	1 mL/minute
Column temperature	40°C	40°C
Rising solvent	Methanol	Acetonitrile
Analysis duration	13 minutes	12 minutes
LOD (ng/kg)	6.18	5
LOQ (ng/kg)	6.50	20
Extraction Yield (%)	98.92±2.49	86±0.39

LOD, Limit of Detection; LOQ, Limit of Quantification

2.6 Statistical analysis

Statistical analyses were performed using SPSS for Window version 20.0 (SPSS Inc., Chicago, Illinois). Analysis of variance (ANOVA) according to two factors: duration and method of storage and Tukey's HSD test at 5% significance level were used to compare the means of physical parameters and mycotoxins levels detected in each granary system. Pearson correlation test was used to assess relationships between the content of moisture, the water activity and mycotoxins levels. Then, Multivariate Analyses through Principal Components Analysis (PCA) and Ascending Hierarchical Clusters analysis (AHC) were performed using STATISTICA software (version 7.1).

3. Results

3.1 Evolution of the physicochemical and mycotoxins parameters

The statistical traits reveal significantly changes ($P<0.05$) in the contents of all parameters assessed according to the duration and the type of storage whether the maize was treated or untreated with biopesticides, except for moisture content and water activity. These parameters were influenced by the factor time alone (Tables II and III).

3.1.1 Water activity

Figures 2 and 3 show the evolution of the water content of maize cobs and grains stored in the three types of granaries. Overall, there was no a significant difference between the types of storage for both maize cobs and grains. The water content in maize (grains and cobs) treated or untreated with *L. multiflora* and *H. suaveolens* evolved in the same direction. At the earlier storage, water content (0.83±0.04) dropping to 0.94±0.03 or 0.92±0.08 in the control and 0.90±0.04 in the traditional and the improved granaries respectively in maize grains and cobs after 8 months of storage.

3.*1.2 Moisture content*

Figures 2 and 3 show the evolution of the moisture content in maize cobs and grains stored in the different granaries. With respective means of 9.23% and 9.05% at the beginning (0 month), the moisture contents increase significantly ($P<0.001$) during the storage period. The highest moisture values are recorded after 8 month of storage in the control granaries with means of 13.82% and 13.52% from maize cobs and grains. These values are higher than the moisture deriving with traditional and improved granaries from both maize cobs (12.85% and 12.74%, respectively) and grains (11.85% and 11.87%, respectively).

3.1.3 Weight loss

The evolution of the weight loss in maize cobs and grains are shown in Figure 2 and 3. With the minimum mean value of 0.02% at the beginning of storage (0 month) for both maize grain and cobs, the percentage of weight loss increase rapidly and significantly in the control granaries, highlighting higher values of 50.80±3.55 % or 60.42±3.10 % from maize cobs or grains after 8 months of storage. But this trait remained constant with time in the treated granaries, presenting little variation until 6 months of storage. Beyond this period, weight loss increase rapidly and showing values from 24.10±2.10 % or 26.10±4 % in the traditional and 21.53±2.52 % or 24.97±2.50 % in the improved granaries.

3.1.4 Free fat acidity, peroxide and iodine values

From the various technologies, the levels of free fatty acids (% FFA) increase significantly ($P<0.05$) during the storage, as a result of hydrolysis of triacylglycerides. The percentage levels of FFA reach at 8 months of storage, a maximum value of 6.00% or 8.68% from maize cobs or grains in the control granaries than the traditional (4.89% or 5.36%) and improved (4.92% or 5.42%) granaries. On the other hand, the peroxide values change steadily with duration and types of storage (Figures 2 and 3). The peroxide values recorded at the earlier storage (2.10 to 2.11 meq O_2/kg) dropped at 5.55% to 9.41% after 8 months of storage, with higher step means from the untreated granaries than the treated ones. Concerning the iodine values, the means of 121.00 or 121.80 g I_2/100g recorded at the earlier storage decrease to 85.40% to 89.00 g I_2/100g with the control and between 99.35 g I_2/100g and 98.00 g I_2/100g for the traditional and improved granaries. Moreover, iodine values from the untreated granaries are lower than values provided by the biopesticides treatments (Figures 2 and 3).

3.1.5 Starches contents

A gradual decrease is observed with the duration of storage (Figures 2 and 3). The starches contents of the maize cobs and grains at the earlier storage (64.90% to 65.10%) drop to 40.25% or 47.80%, to 45.20% or 52.10% and to 46.20% or 51.20% for the control, the traditional and the improved granaries, respectively, after 8 month of storage. Higher step means are recorded from the treated granaries than the untreated ones.

3.1.6 Levels of aflatoxin B_1 and ochratoxin A

The evolution of aflatoxin B_1 and ochratoxin A levels are show in Figures 2 and 3. All maize samples studied were aflatoxin B_1 and ochratoxin A-positive with percentage of 45.83% and 50% of the maize samples of AFB_1 and ochratoxin A levels above the maximum residue limit concentration of 5 µg/kg proposed by the regulations of the European Union (EC, 2006; 2010). The postharvest maize storage revealed a significant increase in the aflatoxins levels ($P<0.05$) during storage, from the beginning till the 8th month.

Considering both maize cobs and grains, the means ranging from 0.28 µg/kg before the storage drop to 121.26 µg/kg or 132.60 µg/kg for the control granaries, to 20.22 µg/kg or 24.60 µg/kg with the traditional granaries and to 23.26 µg/kg or 26.60 µg/kg in the improved granaries. As observed for AFB_1, the 8 months of storage show significant increasing of the ochratoxin A levels involving with the three technologies investigated. The ochratoxin A levels, estimated between 0.26 µg/kg and 0.38 µg/kg before the storage, raise significantly ($P<0.05$) to 18.26µg/kg or 29.26 µg/kg with the traditional granaries, to 16.25 µg/kg or 27.16 µg/kg for the improved granaries and to 58.26 µg/kg or 75.22 µg/kg from the control granaries considering the maize grains or cobs, respectively (Figures 2 and 3). During the storage, the granaries managed with biopesticides allow higher mycotoxins levels to maize than those without any treatment.

3.2 Correlation between the physicochemical and mycotoxins parameters

Values of Pearson indexes (r) showed positive and negative significant links between nine (9) parameters assessed for both maize forms cobs and grains (Table IV and V). Logically the water activity, moisture, free fat acidity, peroxide, iodine, starch, weight loss, aflatoxin B_1 and ochratoxin A were closely correlated during the maize post harvesting storage, r varying from 0.65 to 0.95 for maize cobs and from 0.62 to 0.99 for maize stored as grains. The water activity were directly correlated with the moisture content (r=0.79 and 0.90 for maize grains and cobs respectively), and aflatoxin B_1 and ochratoxin A levels(r=0.62 to 0.66 and 0.62 to 0.76 for maize grains and cobs respectively). Positive and significant relation were observed between the percentage of weight loss and the free fat acidity (r=0.81 and 0.94 for maize cobs and grains respectively) and with peroxide value (r=0.83 and 0.96 for maize cobs and grains respectively). On the other hand, starches contents and iodine values of both maize forms were reversely correlated with all different parameters. Indeed, starches contents, with values of Pearson indexes, ranging negatively and significantly from -0.94 to -0.76 and from -0.95 to -0.89 for maize grains and cobs respectively. Also, Iodine values ranging from -0.94 to -0.87 and from -0.87 to -0.75 for maize grains and cobs respectively

3.3 Variability between storage structures and qualities parameters assessed

Principal Component Analysis (PCA) was achieved with the main factors F1 (Table VI) delivering eigenvalue equal or superior to 1, according to statistical standard of Kaïser. Nevertheless, the component F2 (eigenvalue of 0.33) is associated to F1 for fulfillment of the PCA. Then, gatherings highlighted from the PCA were clarified by Ascending Hierarchical Classification (AHC) performed with the Unweighted Pair Group Method with Arithmetic means (UPGMA). Figure 3 shows the correlation circle between the F1-F2 factorial drawing, which expresses 97.06% of the total variability (Table VI), and parameters assessed for maize stored. All the parameters had significant positive contribution in the formation of F1. The projection of the samples studied highlighted 4 groups of individuals (Figure 4). The Group 1 consists mainly in individuals from control granaries at 8 months of storage which are linked to the characters correlated positively to F1.

Thus, they are characterized by high levels of aflatoxin B_1, ochratoxin A, water activity, moisture content, weight loss, free fat acidity and peroxide value. The second group includes samples resulting from the untreated granaries at 6 months of storage which also overlap with characters correlated positively factor F1. Moreover, these individuals exhibit also high levels of mycotoxins, water activity, moisture content, weight loss, free fat acidity and peroxide value. The third group contains samples from the treated granaries (traditional and improved) at the 8^{th} month of storage. They are distinguished by high levels of mycotoxins, water activity, moisture content, weight loss, free fat acidity and peroxide value during maize conservation. The group 4 is with samples from the treated granaries (traditional and improved) at 2 and 6 months and the control granaries at 2 months of storage, providing high levels of starch content, iodine value and slight levels of mycotoxins, water activity, moisture content, weight loss, free fat acidity and peroxide value than those of other individuals.

The Ascending hierarchical classification (AHC) corroborates the variability observed in the PCA (Figure 5). Indeed, at the gene distance of 24, the UPGMA dendrogram shows four clusters of the maize samples during storage. The first cluster is the control granaries at 8 months of storage with higher levels of AFB1, OTA, water activity, moisture content, weight loss, free fat acidity and peroxide value. The second cluster encloses individuals resulting from the untreated granaries at 6 months of storage, which provide similar parameters to the first cluster. The maize samples deriving from treated granaries at the 8^{th} month of storage inner the third cluster, showing similar high levels of levels of mycotoxins, water activity, moisture content, weight loss, free fat acidity and peroxide value. The fourth cluster includes maize samples from the treated granaries at respective 2 and 6 months and the control at 2 months of storage, which have contents of starches and iodine higher than the values provided by the other individuals.

Table II: Statistical data of physicochemical and mycotoxins parameters of maize cobs storage

Source of Variation	Df		Parameters								
			AW	MC	FFA	PER	IOD	STC	WTL	AFB1	OTA
		SS	0.002	4.23	5.85	5.58	905.43	73.05	601.27	18245.88	5116.28
Types	2	F-value	3.84	6.90	45.00	96.39	22.46	52.62	61.86	1293.80	16.28.85
		P-value	0.04	0.004	<.001	<.001	<.001	<.001	<.001	<.001	<.001
		SS	0.04	83.52	57.16	56.39	2578.91	1045.61	6443.68	20936.91	9907.10
Durations	3	F-value	48.41	90.70	293.11	649.69	42.66	502.15	441.96	1484.62	2102.73
		P-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
		SS	0.002	1.54	2.05	1.92	510.96	26.68	760.46	5537.70	3634.17
Types x Durations	6	F-value	1.17	0.84	5.25	11.08	4.23	6.41	26.08	392.66	385.67
		P-value	0.35	0.56	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Error	24	SS	0.006	7.37	1.56	0.69	483.66	16.66	116.64	338.46	37.69
Total	36	SS	27.52	4876.10	611.37	628.10	449766.02	123205.10	13309.42	1875.37	30916.34

SS, sum of squares; F-value, value of the statistical test; P-value, probability value of the statistical test; df, degree of freedom. MC, moisture content ; FFA, Free fat acidity values ; PER, peroxide values ; IOD, iodine values; STC, starches contents; WTL, weight loss; AFB1, aflatoxin B1 content.

Table III: Statistical data of physicochemical and mycotoxins parameters of maize grains storage

Source of Variation	Df		Parameters								
			AW	MC	FFA	PER	IOD	STC	WTL	AFB1	OTA
		SS	0.004	5.86	17.50	22.51	455.34	81.22	627.23	10276.04	2957.21
Types	2	F-value	2.58	11.72	109.74	208.15	43.05	19.53	146.65	3638.96	501.13
		P-value	0.10	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
		SS	0.04	58.57	98.39	106.71	4612.55	2302.49	4670.23	5606.94	4767.55
Durations	3	F-value	17.27	78.13	411.45	657.93	290.69	369.20	727.95	1985.54	538.61
		P-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
		SS	0.002	6.00	11.16	16.10	180.36	34.67	646.53	2364.27	2037.46
Types x Durations	6	F-value	0.48	1098	23.34	49.64	5.68	2.78	50.39	837.24	115.10
		P-value	0.82	0.11	<.001	<.001	<.001	0.03	<.001	<.001	<.001
Error	24	SS	0.02	6.00	1.91	1.30	126.94	49.90	51.33	67.77	70.81
Total	36	SS	28.20	4527.41	88.35	864.68	393948.73	1097.67	10950.48	7232.67	15841.54

SS, sum of squares; F-value, value of the statistical test; P-value, probability value of the statistical test; df, degree of freedom. MC, moisture content ; FFA, Free fat acidity values ; PER, peroxide values ; IOD, iodine values; STC, starches contents; WTL, weight loss; AFB1, aflatoxin B1 content.

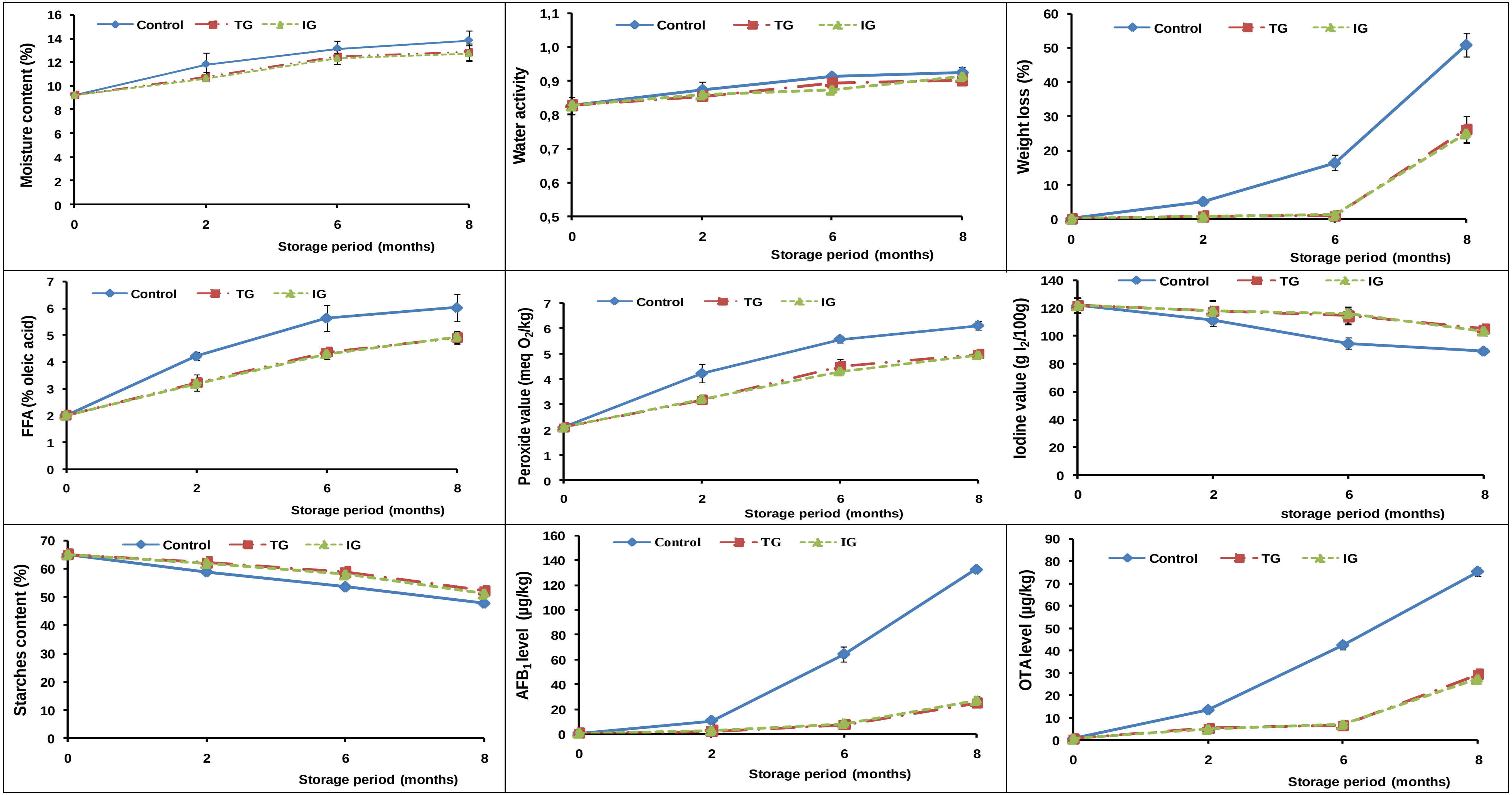


Figure 2: Evolution of physicochemical and mycotoxins parameters of maize cobs according to the storage conditions

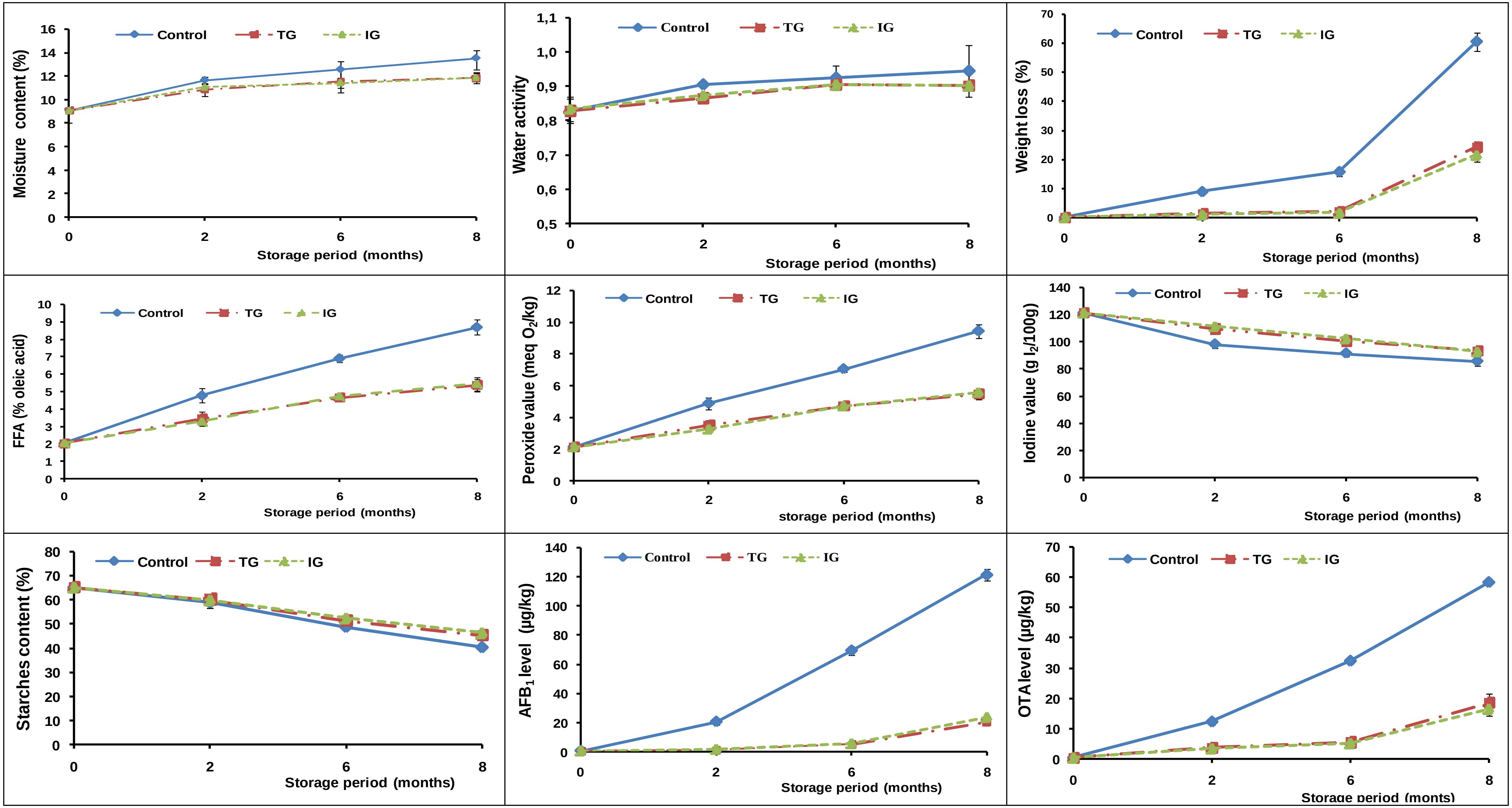


Figure 3: Evolution of physicochemical and mycotoxins parameters of maize grains according to the storage conditions

Table IV: Matrix of correlations between physicochemical and mycotoxins characters of maize grains

	AW	MC	FFA	PER	IOD	STC	WTL	AFB_1	OTA
AW	**1**								
MC	**0.90****	**1**							
FCD	**0.90****	**0.94****	**1**						
PER	**0.92****	**0.95****	**0.99****	**1**					
IOD	**-0.82****	**-0.75***	**-0.87****	**-0.87****	**1**				
STC	**-0.89****	**-0.90****	**-0.94****	**-0.95****	**0.88****	**1**			
WTL	**0.76***	**0.76***	**0.81****	**0.83****	**-0.84****	**-0.92****	**1**		
AFB1	**0.66***	**0.65***	**0.75****	**0.75****	**-0.87****	**-0.73***	**0.75***	**1**	
OTA	**0.76***	**0.74***	**0.83****	**0.84****	**-0.92****	**-0.87****	**0.90****	**0.95****	**1**

*,**= significant at P< 0.05 and 0.01, respectively; AW, Water activity; MC, moisture content ; FFA, Free fat acidity values ; PER, peroxide values ; IOD, iodine values; STC, starches contents; WTL, weight loss; AFB_1, aflatoxin B_1 content.

Table V: Matrix of correlations between physicochemical and mycotoxins characters of maize cobs

	AW	MC	FFA	PER	IOD	STC	WTL	AFB_1	OTA
AW	**1**								
MC	**0.79***	**1**							
FFA	**0.78***	**0.90****	**1**						
PER	**0.81****	**0.90****	**0.99****	**1**					
IOD	**-0.87****	**-0.91****	**-0.94****	**-0.93****	**1**				
STC	**-0.76***	**-0.89****	**-0.94****	**-0.93****	**0.94****	**1**			
WTL	**0.73***	**0.80****	**0.94****	**0.96****	**-0.85****	**-0.91****	**1**		
AFB1	**0.62***	**0.71***	**0.83****	**0.86****	**-0.72***	**-0.70****	**0.82****	**1**	
OTA	**0.62***	**0.76***	**0.91****	**0.92****	**-0.78***	**-0.79****	**0.91****	**0.97****	**1**

*,**= significant at P< 0.05 and 0.01, respectively ; AW, Water activity; MC, moisture content ; FFA, Free fat acidity values ; PER, peroxide values ; IOD, iodine values; STC, starches contents; WTL, weight loss; AFB_1, aflatoxin B_1 content.

Table VI: Eigenvalues and correlation matrices factors of principal components analysis with physicochemical and mycotoxins characters of maize stored studied

Factors	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Eigenvalues	8,41	0,33	0,17	0,06	0,02	0,01	0,00	0,00
Variances (%)	93,41	3,65	1,91	0,72	0,20	0,09	0,01	0,00
cumulative variance (%)	93,41	97,06	98,98	99,70	99,90	99,99	100	100
Aw	-0,96	-0,16	-0,20	-0,03	0,06	0,05	0,00	0,00
Moisture	-0,98	-0,10	-0,12	-0,07	0,01	-0,04	0,02	0,00
FFA	-0,99	-0,02	-0,02	-0,08	-0,05	-0,02	-0,01	-0,01
Peroxide	-0,99	-0,01	0,02	-0,10	-0,05	0,01	0,00	0,00
Iodine	0,98	-0,02	0,12	-0,15	0,07	-0,02	0,00	0,00
Starch	0,95	0,28	-0,09	-0,12	-0,03	0,04	0,01	0,00
weight loss	-0,95	-0,09	0,31	-0,03	0,00	0,03	0,00	0,00
Aflatoxin B_1	-0,93	0,35	0,00	0,00	0,06	-0,01	-0,02	0,00
Ochratoxin A	-0,95	0,29	0,05	0,05	0,00	0,00	0,02	-0,01

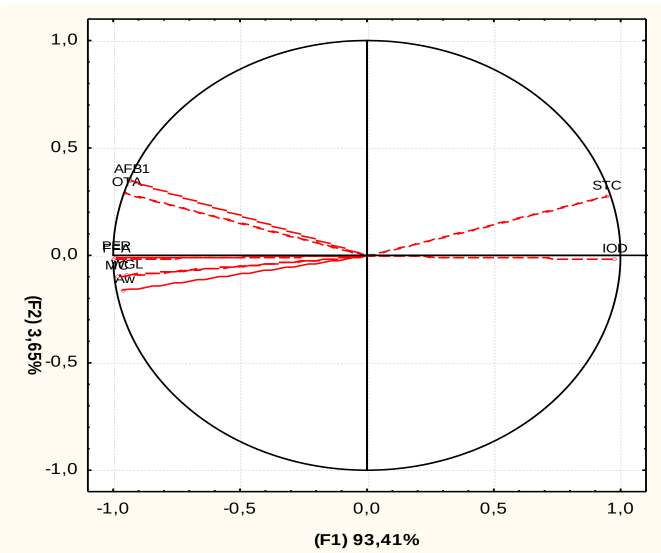


Figure 3: Correlation drawn between the F1-F2 principal components of the parameters deriving from the maize samples studied

AFB_1, aflatoxin B_1 content; **OTA** Ochratoxin A content; **FFA**, free fat acidity content; **PER**, peroxide content; **MC**, moisture content ; **WTL**, weight loss content; **AW**, water activity content, **STC**, starch content; **IOD**, iodine content.

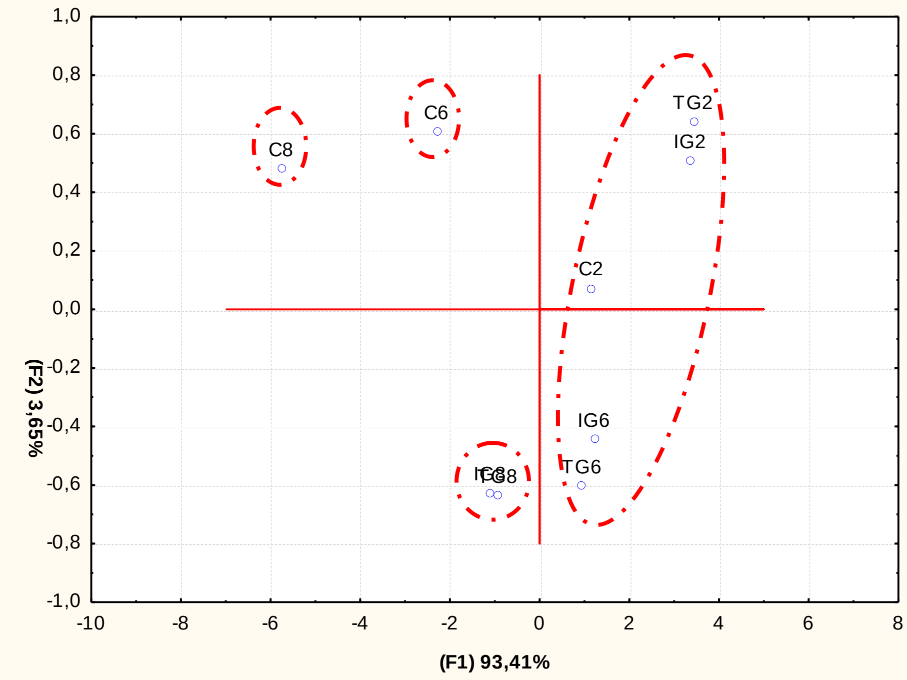


Figure 4: Projection drawn between the F1-F2 principal components of the types of storage deriving from the maize samples studied

C_2, TG_2, IG_2, control, traditional and improved granaries at 2 month of storage;**C_6, TG_6, IG_6**control, traditional and improved granaries at 6 month of storage;**C_8, TG_8, IG_8**control, traditional and improved granaries at 8 month of storage.

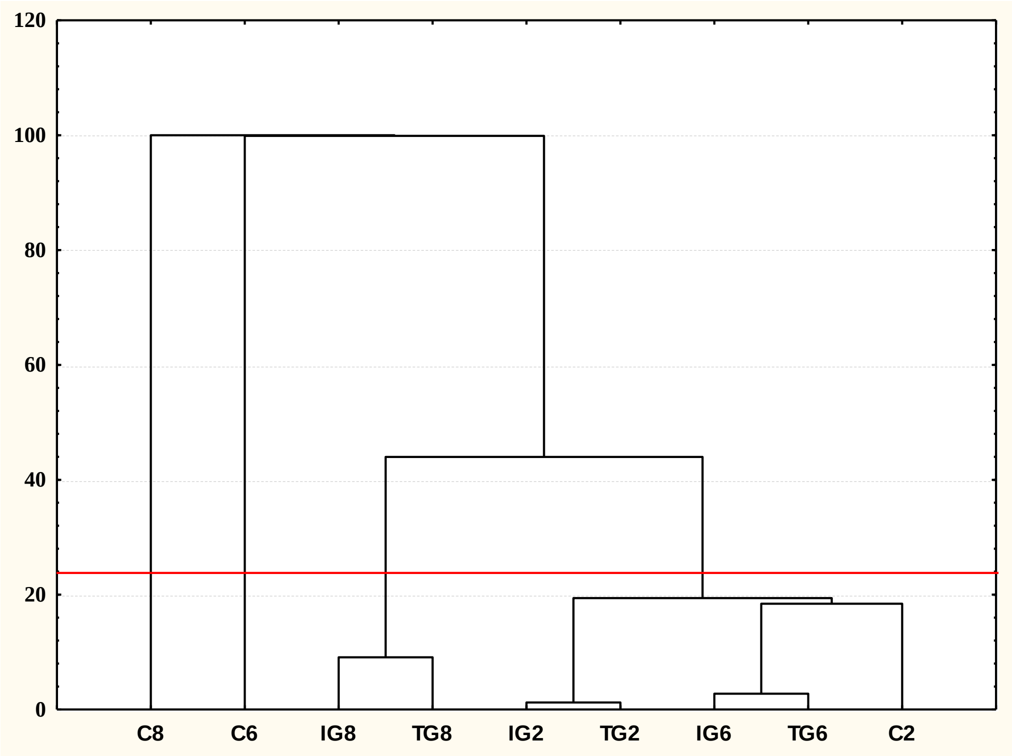


Figure 5 : Ascending hierarchical notation (dendrogram) with the parameters of maize storage

C_2, TG_2, IG_2, control, traditional and improved granaries at 2 month of storage;**C_6, TG_6, IG_6**control, traditional and improved granaries at 6 month of storage;**C_8, TG_8, IG_8**control, traditional and improved granaries at 8 month of storage.

4. Discussion

Three important factors that characterize the maize quality were assessed during storage. It was the merchantable quality includes the water activity, the moisture content and grains weight losses. The nutritive quality includes the rate of free fat acidity, the peroxide and iodine values and the starch content. Hygienic quality includes the aflatoxin B_1 and ochratoxin A levels.

Regardless of the type of storage, granaries cover with the plastic polyethylene film and treated with the two plants could be regarded as a good storing media for maize, because of the low levels of the maize quality recorded compared to controls. Granaries treated with biopesticides at 2 and 6 months are similar to those obtained at 2 months in the untreated granaries. In addition, this attempt shows that the protective property of the combination of the two local plants used is more effective at 6 months of maize storage than at 8 months of storage. These results agree with previous study on the evolution of nutritive compounds of maize stored in granaries with biopesticides (Niamketchi *et al.,* 2016)

The resumption and increase of water activities and moisture contents in granaries storage systems for both maize cobs and grains could be related of respiration of insects and fungi. In fact, moisture is the product of respiration process which increases the moisture content of the stored maize cobs and grains (Chattha *et al.,* 2015). The raise in cobs and grains water activity and moisture content during storage may be also allocated to the relative humidity air, mean of which is around 70%-80% (CNRA, 2014). In fact, few increasing in the relative air humidity above 70% involve with great rising of the moisture content of the stored grains (Di Domenico *et al*., 2015). At the end of storage, both maize cobs and grains presented moisture contents above the limit of 13% recommended for maize safe storage (Mohale *et al.*, 2013). But high water activities recorded in granaries storage systems are more susceptible to spoilage, fungal contamination and rapid mycotoxins production (Schwartzbord *et al.,* 2015). Indeed, strongly correlations were showed between the water activity and nutrive and sanitary parameters assessed.

Free fat acidity, peroxide and iodine values are commonly used as an index of quality deterioration during maize storage because lipid dissolution progresses more rapidly than that of protein and starch (Park *et al*., 2012; Genkawa *et al.,* 2008; Rodriguez-Soana *et al.*,2015). The increase rate of free fat acidity during maize storage maybe due to the increase in grain moisture. The high moisture content of cobs and grains could have raised the hydrolysis and oxidation of lipids, providing a higher free fat acidity value. Our results agree with the works of Paraginski *et al.,* 2014. These authors found a positive correlation between the moisture content, the storage temperature of maize at 5, 15, 25 and 35°C with the fat acidity during 12 months storage. The increase levels of peroxide values recorded during storage could be the result of the hydrolysis and oxidation of lipids regarding on the correlation between fat acidity and peroxide values. Iodine value significantly decreased with respect to the storage duration. The reduction of iodine can be due also to the degradation of lipids involved the oxidation of free fatty acids in the stored maize grains and cobs, consequence of the increase of moisture and molds activities. The increase of free fat acidity may also be due to the insect and fungal attacks in grains during storage (Chattha *et al.,* 2015). The loss of starches contents involves in the rising of the moisture during storage regarding with the reverse correlation between both parameters. The changes of starches contents could result from the grains intrinsic biochemical degradation and/or its needs (Paraginski *et al.,* 2014). Moreover, Chattha *et al* (2015) linked the reduction in the maize starches contents during storage to the part of grains consumed by the associated insects and microorganisms. The reduced starches contents found in our study corroborate the report of simic *et al* (2007) where starches are reduced when exposed to temperature of 25°c for 6 months of storage.

The use of the plastic polyethylene film and the combination of two plants enhance reduction of insect and mycotoxins in stored maize comparing with the control untreated maize. Indeed, the percentage of weight loss and levels of AFB_1 and OTA of the treated maize grains and cobs recorded slight increasing during 6 months storage, when the untreated maize already allowed great pest production. Thus, maize cobs and grain were significantly protected by these treatments from pest infestation up to 6 months in traditional and improved storage granaries. The way of biological action of both plants could result with the release of bioactive molecules involved with the plants leaves oils (N'gamo *et al*., 2007; Tatsadjieu *et al*., 2009). According to Tia (2012), the main bioactive molecules of *L. multiflora* are oxygenated monoterpenes such as linalol and 1,8-cineole; whereas monoterpene hydrocarbons particularly sabinene, β-pinene and limonene predominate from the *H. suaveolens*. The combination of plant materials did also produce significant synergistic or additive effect on inhibition activity against pest growth. Our results corroborate the works of Gueye *et al*. (2013). These authors mentioned the repellent effect of dried leaves of *Hyptis spicigera* and *Hyptis suaveolens* against maize weevil *Sitophilus zeamais* and *Tribolium castaneum* in traditional granaries over a period of 7 months in Kedougou region Eastern Senegal. Ukeh et al (2012) also demonstrated the insecticidal activity of powders to 10% w/w and essential oils of *Aframomum melegueta* and *Zingiber officinale* (Zingiberaceae) which significantly reduce the progeny of maize weevil populations in traditional african granaries over a period of about 3 months in Obudu, southeast Nigeria.

Sharma et al. (2004) showed that the essential oil of *H. suaveolens* has an inhibitory activity on *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus ochraceous* producing mycotoxins such as aflatoxin B_1 and ochratoxin A at level of 500 mg/kg. In addition, study of Tatsadjieu et al. (2009) also showed that the essential oil of *Lippia rugosa*, a species of the genus Lippia, inhibits the growth of *Aspergillus flavus* and limits the production of aflatoxin B1 to an inhibitory concentration of 1000 mg/L. However, the great raising in levels of weight loss beyond 6 months could be due to a decrease repellent activity of the plants materials. Similar observations were made by Liu et al (1999) who explained the rapid drooping in the effectiveness of plants oil-based biopesticides by massive releases of the volatile bioactive molecules in the first days after application.

AFB_1 remain the most potent mycotoxin known, which result carcinogenic, teratogenic, hepatotoxic and immunosuppressive effects on both human and animals (Williams et al, 2004; Liu and Wu, 2010). OTA is also know an important nephrotoxic and nephrocarcinogenic mycotoxin and has been associated with the development of urinary tract tumours in humans.

The coexistence of AFB_1 and OTA in the studied maize stored should be taken into consideration as claimed by the European community (CA, 2011). This is particularly important in regard to possible synergism and additive effects of these mycotoxins. Such co-contamination has been previously observed in maize samples and with other food samples such as wheat, peanut (Kouadio *et al.,* 2014; Boli *et al.,* 2014; Garrido *et al.,* 2012; Sangaré-Tigori et al., 2006; Vrabcheva *et al.,* 2000;). Therefore, in view of the toxicity of AFB1 and OTA, a great deal of effort must be make to eliminate or reduce AFB_1 and OTA content in foods and feedstuffs of the maize by foster best practices of harvesting, drying and storage in order to provide safety to Ivorian people health.

5. Conclusion

The assessment of the merchantable, nutritive and hygienic qualities during the storage of maize showed a continuous degradation of these parameters. The water activity, moisture content, percentage of weight loss, starch content, free fat acidity, peroxide values and iodine values, aflatoxin B1 and ochratoxin A levels were significantly altered during the storage period of the study. This was reflected in increase in water activity, moisture content, weight loss, free fat acidity, peroxide values, AFB_1 and OTA levels and a decrease in starch content and iodine values. Traditional and improved granaries treated with both two local plant species, *Lippia multiflora* and *Hyptis suaveolens* were concluded to be able to ensure proper storage of maize compared to the untreated granaries. This storage technique is inexpensive, easily carried and fits into the millennium guidelines of environment suitability. However, the study needs further investigation to preserve the quality, and ensure healthy and nutritional value of the maize after storage.

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