Migration of Plastic Bag Additives in *Attiéké* (A Fermented Cassava-based Couscous in Côte d'Ivoire)

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Abstract

Attiéké, a couscous made from fermented cassava, is a staple food in Côte d'Ivoire packaged at high temperatures in polythene plastic bags for selling in the Ivorian markets. Antioxidants (BHT and Irgafos168), phthalates (DEP and DIBP) and UV stabilizers (Chimassorb 81 and Chimassorb 944) were analyzed by high performance liquid chromatography both in the plastic bags and in *attiéké* (from the surface to the inside of the *attiéké* ball) at different temperatures in order to study their migration into this foodstuff. Chimassorb 944 migrates almost only on the contact surface between the plastic bags and the *attiéké* ball from 40 to 60°C and up to 1cm at 70°C. However, the other five additives migrate from the surface of the *attiéké* ball up to 1.5 cm inside from 40°C to 70°C after three hours of conditioning. At 30°C, migration is almost zero for all the additives studied.

Keywords: *Attiéké*, Additives, Plastic bags, Migration, Contamination

INTRODUCTION

Oil shortages, health risks such as endocrine disruptions (Yang *et al*., 2011 ; Tranfo *et al*., 2011 ; Hannas *et al*., 2011) and especially environmental problems caused by the use of fossil polymers (non-biodegradable, resistant in nature for about 400 years and causing soil and water pollution) have led several countries around the world including Côte d'Ivoire to ban the production and the use of plastic bags of fossil origin. However, due to a lack of alternative, the packaging of *attiéké* in its plastic bags is still topical. A*ttiéké*, couscous made from fermented cassava, is a staple food in Côte d'Ivoire packaged in polythene plastic bags before being sold on the Ivorian markets. Its annual production is estimated between 18965 tonnes and 40 000 tonnes (Krabi *et al*., 2015). In order to improve the physicochemical properties of the bags, during their synthesis, several additives are incorporated into them, namely: Antioxidants such as BHT (2,6-di-tert-butyl-p-cresol) and Irgafos 168 (phosphorousacid, tris(2,4-di-ter-butylphenyl) ester), phthalates such as DEP (diethylphthalate) and DIBP (diisobutylphthalate) and UV stabilizers, Chimassorb 81 (2-hydroxy-4-n-octyloxybenzophenone) and Chimassorb 944 (Poly-{6-[1,1,3,3-tetramethylbutyl]-amino]-1,3,5-triazine-2,4-diyl}{2-(2,2,6,6-tetramethylpiperidyl)-amine}). Thus, we could notice the contamination of the *attiéké* by these substances contained in its packaging bags. Haitao *et al.* (2015) studies revealed the migration of antioxidant 2,6-di-tert-butyl-p-cresol (BHT) from high-density polythene packaging into food simulant. Miriany *et al.* (2014) showed the migration of phthalates from plastics to food simulants during microwave cooking. From the above, it would be interesting to study the migration of additives from plastic bags into *attiéké*. This will involve studying the packaging of the *attiéké* at different temperatures in the plastic bags used as packaging for the *attiéké*.

2. MATERIALS AND METHODS

2.1 Equipment

Experimental procedures for detecting and quantifying antioxidants, phthalates and UV stabilizers in *attiéké* are based on the ASTM D 6953-2011 standard and studies conducted by Chengfa *et al.* (2014), Lahimer *et al.* (2013) and Kombo *et al.* (2019).

2.1.1 Study material

The materials studied are plastic bags sold in markets and *attiéké* (couscous made from fermented cassava).

2.1.2 Apparatus and chemicals

The qualitative and quantitative analysis of additives is carried out by a SCHIMADZU high-performance liquid chromatography chain. This line consists of a TRAY tank, DGU-20A5 degasser, SIL-20A autosampler, LC-20AT pump, CTO-20A oven and SPD-20A UV-VIS detector. The software used for data acquisition is LC solution. The separation of these 6 substances was done with a C18 column (Supelcosil LC 18, 15cm x 4.6 mm, 5μm). The BHT, DEP and DIBP were purchased from Sigma-Aldrich. Irgafos 168 and Chimassorb 944 were obtained from Ciba Specialty Chemicals. For n-hexane and toluene, they were supplied by Merck. The Chimassorb 81 was purchased from TCI America. Acetonitrile and methanol for HPLC were provided by BDH Laboratory Supplies.

2.2 Methodology

2.2.1 Sampling of plastic bags

The *attiéké* producers and *attiéké* sellers in detail from the nine municipalities of the city of Abidjan (Abobo, Adjamé, Attécoubé, Cocody, Koumassi, Marcory, Port-Bouet, Treichville and Yopougon) were investigated (Krabi *et al.*, 2015). A total of 90 people (producers and saleswomen) were surveyed to find out what type of bags they use to package the *attiéké*. These surveys mainly identified plastic bags from six factories located in the industrial areas of Yopougon and Koumassi. Thus, sampling was about 120 plastic bag samples from these six plants at the rate of 20 bags per plant. Among these six (6) plants, three (3) have the most used bags: plant1, plant2 and plant3.

2.2.2. A sampling of the *attiéké*

Three-stage cluster sampling of units was used. At the primary level, the cities selected are Dabou, Jacqueville and Abidjan. Located in the south of Côte d'Ivoire, they are known to be the main producers of *attiéké*. At the secondary level, four municipalities in the city of Abidjan (Abobo, Attécoubé, Cocody and Marcory), 1 village in the city of DABOU and 1 village in the city of JACQUEVILLE. *Attiéké* production sites constitute the tertiary level (Krabi *et al.*, 2015). *Attiéké* type 1 "small grain"; was collected from production sites in Dabou, Jacqueville and Aboboté in the municipality of Abobo. As for the *attiéké* type 2 "big grain"; called *agbodjama*, it was collected from production sites in Anono in the commune of Cocody, Anoumambo in the commune of Marcory and Abobodoumé in the commune of Attécoubé. At each site, approximately 200g of *attiéké* were collected (Figure 1). Samples were taken at 70°C, 60°C, 50°C, 40°C and 30°C. For each temperature, 9 samples were taken and packaged in plastic bags from plant1 (3), plant 2 (3) and plant 3 (3) that produce the most used bags. After each conditioning period (1 hour, 2 hours and 3 hours), using stainless steel pliers, 5 g of *attiéké* were collected at different levels of *attiéké* ball depths: at the surface (up to 0.5 cm), at 1 cm and at 1.5 cm (figure 2). A total of 45 *attiéké* balls were collected from each site and 270 *attiéké* balls were collected from all 6 sites. The control consists of the *attiéké* taken under the same conditions and introduced into a brown glass vial (Kombo *et al.*, 2019).

Figure 1: *Attiéké* packaged into the plastic bag

Figure 2: *Attiéké* sample collection at different levels (surface and inside) using stainless steel pincers

2.2.3 Determination of additives

2.2.3.1. Preparation of standard solutions

Ten milligrams of each compound are introduced into 10 mL of methanol to obtain solutions with a concentration of 1000 mg/L. The standard solutions of each substance were prepared by a series of dilutions in acetonitrile at 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0 mg/L. All its solutions are stored in brown glass vials at 4°C (Bo *et al.*, 2014).

2.2.3.2. Mobile phase

Table 1 records the percentages of the mobile phase A (acetonitrile) and the mobile phase B (water) as a function of the duration of analysis (Gao *et al.*, 2013).

Table 1: Percentages of mobile phases A and B according to the duration of the analyses Time (minutes) Acetonitrile Water

Duration (minutes)	Acetonitrile	Water
0	65	35
3	65	35
20	95	5
30	95	5
31	65	35
35	65	35

2.2.3.3. Extraction protocol of additives from plastic bags

Two grams of plastic bags are cut into thin strips and placed in an Erlenmeyer. Fifty millilitres of toluene are added. The mixture is heated to about 80°C and stirred manually until the polymer is completely dissolved. A hundred (100) mL of methanol is then introduced to precipitate the polymer (Li *et al.*, 2017). The mixture is filtered twice using a Whattman grade 5 filter paper. The solvent is evaporated using a rotary evaporator at 70°C at 150 rpm (Lahimer *et al.*, 2013). The volume is reduced to the last drop. This drop is then diluted with 1 mL acetonitrile and analyzed by HPLC (Chengfa et al., 2014).

2.2.3.4. Extraction protocol of additives from *attiéké*

The sample preparation is done according to ASTM D 6953 - 11 with some modifications. In a 125mL flat-bottomed flask, five grams of *attiéké* are introduced. Then 50 mL of n-hexane is added using a pipette. A magnetic bar is also introduced to homogenize the mixture. To improve extraction, two grams of sodium chloride are also added (Chengfa *et al.*, 2014). The mixture is brought to a boil for one hour in a reflux system. Cooling is done at room temperature by removing the heated magnetic stirrer and holding the reflux device. The mixture is filtered (with Whatman grade 5 filter paper) under vacuum using a Büchner. Ten millilitres of n-hexane are added to the solid residue and filtered (this operation is repeated 3 times). The filtrate is concentrated using a rotary evaporator at a rate of 150 rpm at a temperature of 70°C (Lahimer *et al.*, 2013). The volume is reduced to the last drop and this drop is then diluted with 1 mL acetonitrile and analyzed by HPLC (Chengfa *et al.*, 2014).

2.2.3.5. Chromatographic conditions

A UV detector with a wavelength of 224 nm is used for detection. The flow rate is 1.00 mL/min and the analysis time is 40 minutes. The injection volume is 20μL. All analyses were performed at 30°C under isocratic conditions.

2.2.3.6. Detection limit / Quantification limit

The detection limit and quantification limit of the method are obtained by the signal-to-noise ratio of 3 and 10 times, respectively from the baseline of the blank matrix (CEAEQ, 2013). The blank matrix was prepared from the peeled cassava tubers, washed in distilled water and then crushed with a pestle and porcelain mortar. This matrix is processed according to the *attiéké* additive extraction protocol described above. Detection and quantification limits are recorded in Table 2 (Kombo *et al.*, 2019).

Table 2: Limit of detection (LOD) and limit of quantification (LOQ) of the different compounds

Compounds	LOD (mg/Kg)	LOQ (mg/Kg)
BHT	0.32	1
Irgafos 168	0.7	2.1
DEP	0.35	1.1
DIBP	0.06	0.18
Chimassorb 81	0.43	1.3
Chimassorb 944	0.82	2.5

2.2.3.7. Linearity

It is the ability to obtain results within an interval that are directly proportional to the concentration of the substance analyzed in a sample. It was tested through linear regression using XLSTAT statistical software version 2014. The model equations, determination coefficients and linearity range of the different additives are recorded in Table 3 (Kombo *et al.*, 2019).

Table 3 : The model equation, the determination coefficient (R^2) and the linearity range of the different compounds

Compounds	Compounds Model equation (y is the concentration in mg/L and x the peak area in area units)	R^2	Linearity range (mg/L)
BHT	$y = 0.822699596011649 + 1.19592678718014 \times 10^{-2} x$	0.9975	1.0 – 100.0
Irgafos 168	$y = -0.490609398053458 + 1.81395998477025 \times 10^{-2} x$	0.999	2.0 – 100.0
DEP	$y = 0.377747863732544 + 2.46646299321994 \times 10^{-2} x$	0.9992	0.1 – 100.0
DIBP	$y = -0.963896971408552 + 1.65718898455463 \times 10^{-2} x$	0.9992	0.2 – 100.0
Chimassorb 81	$y = -0.242388605780558 + 1.40142106737983 \times 10^{-2} x$	0.9985	1.0 – 100.0
Chimassorb 944	$y = -0.241252050458893 + 5.27974981711409 \times 10^{-2} x$	0.9984	2.0 – 100.0

2.2.3.8. Correlation test of different additives

The correlation test makes it possible to demonstrate a dependence between the various additives.

3. RESULTS

The chromatogram of the standard solutions obtained under the operating conditions described is shown in Figure 3, which clearly distinguishes the retention times of the different compounds. Besides, the injection of n-hexane did not give any peak to the retention time of the desired compounds, which shows the absence of memory effect.
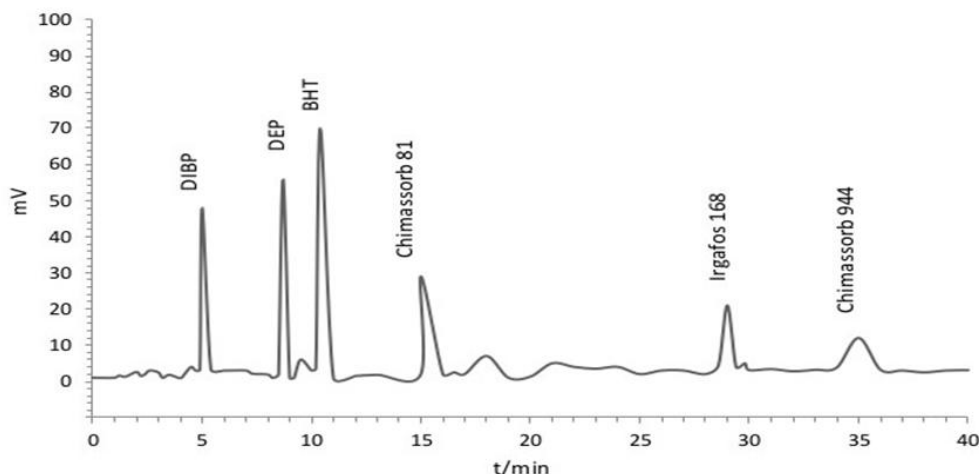

Figure: 3 Chromatogram of the different compounds at 10 mg/L

3.1. Analysis of antioxidants, phthalates and UV absorbers in plastic bags

Except DEP, which is absent in the sample from plant 5, all other dosed additives are present in all samples from the different plants. Indeed:
- the concentration of BHT is high in the bag samples from plants 1, 3 and 5 and low in those from plant 2;
- The concentration of Irgafos 168 is high in the bag samples from plants 3 and 5 and low in those from plants 2 and 4;
- The lowest average concentration of DEP (23 mg/Kg) is that of bags from Plant 3 and the highest is that of bags from Plant 4 (51 mg/Kg);
- The lowest average concentration of DIBP (47 mg/Kg) is that of bags from plant 4, while the highest (89mg/Kg) is that of plant 6;
- The concentrations of Chimassorb 81 and Chimassorb 944 are low in the bags of plant 6 while those of plant 2 has higher contents.
All its values are indicated below in Table 4.

Table 4: Analysis of antioxidants, phthalates and UV absorbers in plastic bags

	Concentration in mg/Kg					
	BHT	Irgafos 168	DEP	DIBP	Chimassorb81	Chimassorb944
Plant 1	26±2.01	22.6±1.32	45±3.53	53±2.51	40.95±3.98	22.4±0.95
Plant 2	18±1.51	14.3±0.77	34±3.63	65±4.83	48±4.56	29.4±1.42
Plant 3	24±0.95	23.4±0.87	25±2.51	70±5	42±3.98	16.7±0.89
Plant 4	21±1.22	16.8±1.27	51±4.51	47±3.47	40±2.51	17.9±1.22
Plant 5	25±1.91	24.1±2.14	nd	80±5.01	34±3.11	20.3±1.97
Plant 6	19.87±1.2	20.1±2.05	48±4.1	89±4.84	21±2.81	14.6±0.68

nd= not detected

3.2. Analysis of antioxidants, phthalates and UV absorbers in control *attiéké*

The additives analysed in the control *attiéké* gave the results reported in Table 5. BHT, Irgafos 168, Chimassorb 81 and Chimassorb 944 were not detected. However, DEP and DIBP have been identified and quantified. The mean values of the different DEP and DIBP results vary between 1.185 and 2.444 mg/Kg and between 0.18 and 2.1 mg/Kg respectively. The highest concentration of DEP was obtained with the Aboboté *attiéké* while the Dabou *attiéké* had the lowest level. As for DIBP, the lowest level was obtained with Jacqueville's *attiéké* and the highest with Abobodoumé's *attiéké*.

Table 5: Concentration of the different substances in the control *attiéké*

	The concentration of compounds ± standard deviation (mg/Kg)					
Additives	**DABOU**	**JACQUEVILLE**	**ABOBOTE**	**ANONO**	**ABOBODOUME**	**ANOUMAMBO**
BHT	nd	nd	nd	nd	nd	nd
Irgafos 168	nd	nd	nd	nd	nd	nd
DEP	1.185 ±0.167	1.265 ± 0.146	2.444 ± 0.466	1.5 ± 0.209	1.79 ± 0.147	1.4 ± 0.221
DIBP	0.97 ± 0.217	0.18 ± 0.03	1.678 ± 0.2	0.766±0.92	2.1 ± 0.61	1.92 ± 0.32
Chimassorb81	nd	nd	nd	nd	nd	nd
Chimassorb944	nd	nd	nd	nd	nd	nd

3.3. Analysis of antioxidants, phthalates and UV absorbers in *attiéké* packaged in plastic bags

Overall, the concentrations of the additives decrease from the surface to inside the *attiéké* ball. However, they increase according to packaging time duration and temperature.

3.3.1. Analysis of bag additives in *attiéké* at 70°C

At a temperature of 70°C, the highest average BHT concentration is 3.46 mg/Kg on the surface of the *attiéké* ball after 3 hours of conditioning, while inside the ball (at 1.5 cm), after one hour, the concentration is the lowest (1.21 mg/Kg). Irgafos168, DEP, DIBP, Chimassorb 81 and Chimassorb 944 have average concentrations of 5.22, 2.71, 2.03, 4.4 and 5.07mg/Kg respectively on the surface of the *attiéké* ball after 3 hours of conditioning. At this level, concentrations are the highest while after 1 hour of conditioning, at 1.5 cm from the surface of the ball, the lowest values are observed. All these values are mentioned in Table 6.

Table 6: Analysis of additives in *attiéké* packaged in plastic bags at 70°C

		Additives in mg/Kg					
Duration	**Position**	**BHT**	**Irgafos168**	**DEP**	**DIBP**	**Chimassorb81**	**Chimassorb944**
1h	0.5 cm	3.22 ± 0.29	4.71 ± 0.87	2.6 ± 0.52	2 ± 0.69	3.98 ± 0.75	4.68 ± 0.88
	1 cm	1.84 ± 0.31	3.2 ± 0.54	2.28 ± 0.42	1.58 ± 0.66	1.87 ± 0.49	2.83 ± 0.33
	1.5 cm	1.21 ± 0.09	2.11 ± 0.01	1.87 ± 0.38	1.43 ± 0.64	1.28 ± 0.05	nd
2h	0.5 cm	3.38 ± 0.32	5.12 ± 0.94	2.65 ± 0.52	2.02 ± 0.72	4.28 ± 0.71	4.94 ± 0.76
	1 cm	1.98 ± 0.29	3.17 ± 0.71	2.33 ± 0.42	1.65± 0.68	1.99 ± 0.6	2.98 ± 0.47
	1.5 cm	1.26 ± 0.06	2.15 ± 0.05	1.9 ± 0.38	1.46 ± 0.64	1.31 ± 0.05	nd
3h	0.5 cm	3.46 ± 0.31	5.22 ± 0.97	2.71 ± 0.52	2.03 ± 0.72	4.4 ± 0.73	5.07 ± 0.77
	1 cm	2.04 ± 0.3	3.25 ± 0.76	2.36 ± 0.42	1.65 ± 0.7	2.07 ± 0.66	2.93 ± 0.46
	1.5 cm	1.34 ± 0.13	2.26 ± 0.31	1.93 ± 0.38	1.49 ± 0.65	1.34 ± 0.05	nd

3.3.2. Analysis of bag additives in *attiéké* at 60°C

At 60°C the BHT concentration increases to 2.85 mg/Kg on the surface of the *attiéké* ball after 3 hours of conditioning while inside the ball (at 1.5 cm), the increase is only 1.07 mg/Kg. Irgafos168, DEP, DIBP, Chimassorb 81 and Chimassorb 944 reach the respective concentrations 4.5, 2.38, 1.93, 3.4 and 3.47 on the surface of the *attiéké* ball after 3 hours of conditioning. The lowest values are those obtained after 1h of conditioning at 1.5 cm from the surface of the ball. All these values are recorded in Table 7.

Table 7: Analysis of additives in *attiéké* packaged in plastic bags at 60°C

		Additives in mg/Kg					
Duration	**Position**	**BHT**	**Irgafos168**	**DEP**	**DIBP**	**Chimassorb81**	**Chimassorb944**
1h	0.5 cm	2.55 ± 0.27	376 ± 0.75	2.3 ± 0.5	1.88 ± 0.68	3.15 ± 0.77	3.23 ± 0.46
	1 cm	1.49 ± 0.12	2.26 ± 0.17	1.94 ± 0.44	1.51 ± 0.61	1.53 ± 0.21	nd
	1.5 cm	nd	nd	1.7 ± 0.41	1.34 ± 0.68	nd	nd
2h	0.5 cm	2.76 ± 0.23	4.36 ± 0.86	2.35 ± 0.5	1.91 ± 0.69	3.41 ± 0.81	3.37 ± 0.48
	1 cm	1.51 ± 0.27	2.62 ± 0.29	2 ± 0.44	1.55 ± 0.65	1.58 ± 0.2	nd
	1.5 cm	nd	nd	1.69 ± 0.4	1.38 ± 0.68	nd	nd
3h	0.5 cm	2.85 ± 0.25	4.5 ±0.89	2.38 ± 0.52	1.93 ± 0.7	3.49 ± 0.81	3.47 ± 0.57
	1 cm	1.72 ± 0.51	2.68 ± 0.28	2.04 ± 0.44	1.55 ± 0.62	1.56 ± 0.23	nd
	1.5 cm	1.07 ± 0.05	2.12 ± 0.01	1.71 ± 0.4	1.39 ± 0.68	traces	nd

3.3.3. Analysis of bag additives in *attiéké* at 50°C

At 50°C, at a distance of 1.5 cm from the surface of the *attiéké* ball, until 2 hours, BHT, Irgafos 168, Chimassorb 81 and Chimassorb 944 are not detected in the *attiéké*. However, after a conditioning time of 3 hours, these additives are quantified. Table 8 shows that the highest concentrations of additives are obtained on the surface of the ball after 3 hours of conditioning while the lowest concentrations are obtained after one hour at 1.5 cm inside the *attiéké* ball.

Table 8: Analysis of additives in attiéké packaged in plastic bags at 50°C.

Duration	Position	Additives in mg/Kg					
		BHT	**Irgafos168**	**DEP**	**DIBP**	**Chimassorb81**	**Chimassorb944**
1h	**0.5 cm**	1.82 ± 0.43	2.78 ± 0.41	208 ± 0.46	1.72 ± 0.64	2.08 ± 0.62	2.57 ± 0.08
	1 cm	Traces	2.11 ± 0.01	1.7 ± 0.41	1.37 ± 0.65	nd	nd
	1.5 cm	nd	nd	1.61 ± 1.29	1.29 ± 0.67	nd	nd
2h	**0.5 cm**	1.87 ± 0.44	2.5 ± 0.57	2.11 ± 0.49	1.78 ± 0.65	2.22 ± 0.65	2.59 ± 0.09
	1 cm	1.03 ± 0.05	2.13 ± 0.05	1.73 ± 0.42	1.38 ± 0.65	1.38 ± 0.1	nd
	1.5 cm	nd	nd	1.6 ± 0.45	1.29 ± 0.67	nd	nd
3h	**0.5 cm**	1.91 ± 0.44	3 ± 0.58	2.12 ± 0.51	1.79 ± 0.67	2.31 ± 0.75	2.61 ± 0.11
	1 cm	1.08 ± 0.03	2.15 ± 0.06	1.75 ± 0.43	1.4 ± 0.66	1.41 ± 0.12	nd
	1.5 cm	nd	traces	1.62 ± 0.43	1.3 ± 0.67	traces	nd

3.3.4. Analysis of bag additives in *attiéké* at 40°C

Table 9 shows at 40°C, BHT, Irgafos 168 and Chimassorb 81 is only detected at the surface of the *attiéké* ball regardless of the weather. Chimassorb 944 is present at the surface after 2 hours of conditioning. The highest concentrations for BHT, Irgafos 168, Chimassorb 81 and Chimassorb 944 are 1.43, 2.23, 2.64 and 2.63 mg/Kg respectively after three hours of conditioning. As for the DEP and DIBP, they have average concentrations that increase very slightly (respectively from 1.66 to 1.68 mg/Kg and from 1.32 to 1.34 mg/Kg) on the surface of the *attiéké* ball from 1h to 3h.

Table 9: Analysis of additives in attiéké packaged in plastic bags at 40°C

Duration	Position	Additives in mg/Kg					
		BHT	**Irgafos168**	**DEP**	**DIBP**	**Chimassorb81**	**Chimassorb944**
1h	0.5 cm	1.33 ± 0.22	2,12 ± 0,02	1.66 ± 0.42	1.32 ± 0.71	1.54 ± 0.2	2,55 ± 0,05
	1 cm	nd	nd	1.6 ± 0.44	1.27 ± 0.7	nd	nd
	1.5 cm	nd	nd	1.6 ± 0.44	1.27 ± 0.7	nd	nd
2h	0.5 cm	1.39 ± 0.22	2.21 ± 0.25	1.67 ± 0.42	1.33 ± 0.69	1.65 ± 0.27	2.63 ± 0.09
	1 cm	nd	nd	1.6 ± 0.44	1.27 ± 0.7	nd	nd
	1.5 cm	nd	nd	1.6 ± 0.44	1.27 ± 0.7	nd	nd
3h	0.5 cm	1.43 ± 0.24	1.68 ± 0.42	1.34 ± 0.69	1.68 ± 0.42	2.64 ± 0.11	nd
	1 cm	nd	nd	1.6 ± 0.44	1.27 ± 0.7	nd	nd
	1.5 cm	nd	nd	1.6 ± 0.44	1.27 ± 0.7	nd	nd

3.3.5. Analysis of bag additives in *attiéké* at 30°C

Table 10 shows that at 30°C, generally, only the DEP and DIBP are present in the *attiéké* ball from the surface to the interior. Concentrations remain constant whatever the position of sampling of the *attiéké* sample and packaging time length. It is 1.6 ± 0.44 mg/Kg for DEP and 1.27 ± 0.7 mg/Kg for DIBP.

Table 10: Analysis of additives in *attiéké* packaged in plastic bags at 30°C

Duration	Position	Additives in mg/Kg					
		BHT	**Irgafos168**	**DEP**	**DIBP**	**Chimassorb81**	**Chimassorb944**
1h	0.5 cm	nd	nd	1.6 ± 0.44	1.27 ± 0.7	traces	nd
	1 cm	nd	nd	1.6 ± 0.44	1.27 ± 0.7	nd	nd
	1.5 cm	nd	nd	1.6 ± 0.44	1.27 ± 0.7	nd	nd
2h	0.5 cm	nd	nd	1.6 ± 0.44	1.27 ± 0.7	traces	nd
	1 cm	nd	nd	1.6 ± 0.44	1.27 ± 0.7	nd	nd
	1.5 cm	nd	nd	1.6 ± 0.44	1.27 ± 0.7	nd	nd
3h	0,5 cm	nd	nd	1.6 ± 0.44	1.27 ± 0.7	1.36 ± 0.06	traces
	1 cm	nd	nd	1.6 ± 0.44	1.27 ± 0.7	nd	nd

3.4. Correlation test of the different plastic bag additives

In Table 11, the positive and negative correlation coefficients between the different additives in plastic bags are observed. High positive coefficients are observed between BHT and Irgafos168 (0.8815) and between Chimassorb 81 and Chimassorb 944

(0.7138).

Table 11: Correlation matrix of the different additives in plastic bags

	BHT	**Irgafos 168**	**DEP**	**DIBP**	**Chimassorb81**	**Chimassorb944**
BHT	1					
Irgafos 168	0.8815	1				
DEP	-0.4038	-0.5135	1			
DIBP	-0.1214	0.3333	-0.4294	1		
Chimassorb81	0.0180	-0.3262	-0.0805	-0.6963	1	
Chimassorb944	-0.2466	-0.4963	-0.1291	-0.3246	0.7138	1

4. DISCUSSION

Antioxidants (BHT and Irgafos168), phthalates (DEP and DIBP) and UV stabilizers (Chimassorb 81 and Chimassorb 944) were detected and quantified in the plastic bags because these chemical compounds are additives used in the formulation of the plastic bags. In fact, Irgafos 168 and BHT are thermal antioxidants that are added to plastic polymers to protect them from thermo-oxidation during manufacturing. The correlation coefficient determined between BHT and Irgafos168 (0.8815) confirms the simultaneous use of these two thermal antioxidants. As for plasticisers (DEP and DIBP), their role is to soften plastic polymers (Bhunia *et al.*, 2013). In order to stabilize polymers against UV rays from the sun. Chimassorb 81 is often associated with HALS (Hindered-Amine Light Stabilizers) (Cristofoli and *al*., 2012). According to Cheloufi *et al.* (2014), the simultaneous incorporation of Chimassorb 81 and Chimassorb 944 even at low doses (0.1%) increases the crystallinity rate of low-density polyethylene by 34.03%. This feature further improves the physicochemical properties of the polymer. This relationship is confirmed by the correlation coefficient (0.7138) between these two substances. The average concentrations of BHT determined in plastic bags vary between 18 and 26 mg/Kg, those of Irgafos 168 between 14.3 and 24.1 mg/Kg and those of Chimassorb 81 between 21 and 48 mg/Kg. Li *et al.* (2017) did not detect BHT in polypropylene bags in China; this could be due to the use of another primary antioxidant in the formulation. However, they determined Irgafos 168 at mean concentrations between 166.47 mg/Kg and 845.27 mg/Kg, higher than those determined in this study. For Chimassorb 81, the average values determined are higher than those of Li *et al.* (2017) (5.08 mg/Kg) but lower than those of Gillet (2008) in the industrial formulation of high density polyethylene (668 mg/Kg). Chimassorb 944 in plastic bags was quantified at average concentrations ranging from 14.6 to 29.4 mg/Kg. These concentrations are much lower than Gillet (2008) formulation, which averaged 3327 mg/Kg in high-density polyethylene films. All these differences can be explained by the difference in formulation for the manufacture of finished products and also by the nature of the raw materials used. The highest determined DEP concentration is 51 mg/Kg. This concentration is high compared to that found in low-density polyethylene and high-density polyethylene bags in Ghana by Ayamba (2015).

Indeed, this author determined the presence of other phthalates in these plastic bags such as DEHP (diethylhexylphthalate) and DBP (dibutylphthalate) at average concentrations of 5.84 mg/Kg and 9.94 mg/Kg respectively. This could justify the differences between the different values. The average concentrations of DIBP, which range from 14.6 to 29.4 mg/Kg in the study bags, are significantly lower than those determined in soft polyvinyl chlorides (PVC) (between 16000 and 354000 mg/Kg) (Nilsson *et al.*, 2016). This difference is normal because the concentrations of phthalates are much higher in PVC than in polyethylene bags (Nilsson *and al.*, 2016).

With the exception of DEP and DIBP which were quantified in the control *attiéké* (not packaged in plastic bags), all other substances were not detected. Indeed, phthalates, although synthetic, are used in several fields (cosmetics, plastics, paints, inks, medicines, school materials, etc.). As a result, they have become ubiquitous. The contamination was certainly made during some stages of the *attiéké* manufacturing process such as storage for two to three days in jute bags, spin drying in nylon bags, drying on plastic bags. . . (Yéboué *et al.*, 2017; Kombo *et al.* , 2019).

The migration rate of BHT was highest in the parts in contact between the bag and the *attiéké* at 70°C after 3 hours of conditioning (3.46 mg/Kg). This migration situation is similar to that found by Alavi *et al.* (2015) in the migration of BHT from plastic bags to soybean oil. In this study by Alavi *et al.* (2015), the BHT level reached 42 mg/L after 15 minutes in a 100 Watt microwave oven. As for Irgafos 168, after 1 hour of conditioning at 70°C, the migration rate was on average 4.71 mg/Kg on the surface of the *attiéké* ball. This migration situation of irgafos 168 at a certain temperature was also observed to some extent in the study by Alavi *et al* (2015); the rate of Irgafos 168 migrating after only 2 minutes of contact with olive oil in a 100 Watts microwave oven was 27 mg/L. The values determined by these authors in 2015 are higher than those in this study because the temperature is certainly higher in the microwave oven and the migration is more pronounced in fats (Hadjmohammadi *et al*, 2012). *Attiéké*, on the other hand, is a low-fat commodity (0.64%) (Yeboué *et al.*, 2017). At 40°C, low migration rates are observed at the contact surface between the bags and the *attiéké* ball. GAO *et al.* (2011) have shown that BHT migrates from plastic bags to aqueous simulants and also to fat at 40°C for 10 days.

Ayamba (2015) showed that DEP migrates from plastic bags into olive oil at rates of 1.04 mg/Kg at 80°C after 2 hours of contact. This concentration is similar to that determined in this study at 70°C after the same time in the contact zone with the sachet (1.05 mg/Kg). This similarity of results is due to the fact that migration is more pronounced in fatty or acidic foods (Greta, 2018) such as *attiéké* which has a pH of about 4.7 (Yeboué *et al.*, 2017). Simoneau *et al.* (2011) determined a migration rate of DIBP (0.05 mg/Kg) from the plastic bottle into ethanol at 50% at 70°C for two hours. This concentration is lower than that detected in this study at the same temperature and duration (on average 0.751 mg/Kg). Several factors can explain this: the initial

concentration of DIBP in the plastic polymer, the stiffness of the bottle compared to the plastic bags and also the acidity of the *attiéké*. In addition, low-density polythene is a plastic polymer with the highest diffusion capacity (Bott *et al.*, 2014).

Chimassorb 81 migrated up to 4.28 mg/Kg at 70°C into the *attiéké* after 2 hours of time in the part in contact with the bag. However, after the same contact time, the migration rate of this additive to olive oil at 175°C was 7.6 mg/Kg in the terephthalate polythene (TEP) contact area (Begley *et al.*, 2005). The higher concentration of Chimassorb 81 found by Begley *et al.*, (2005) can be explained by the higher temperature (175°C).

Chimassorb 944 hardly migrates inside the *attiéké* ball; this could be due to its structure (steric clutter) (BASF, 2016) and its large size (Regina *et al.*, 2009). According to Dopico-Garcia *et al.* (2012), low molecular weight HALS diffuse faster than the high molecular weight HALS to which Chimassorb 944 belongs; hence their preferred use by industry. At 30°C, the migration of all the additives analysed is almost nil under the conditions of the study. On the whole, concentrations increase according to packaging duration and temperature. However, they decrease from the surface to inside the *attiéké* ball. Similar trends were observed by Musoke *et al.* (2015), when studying the migration of chemical contaminants from polythene plastic bags into "posho" flower corn meal-based food during cooking. Haitao *et al.* (2015) also demonstrated that the migration rate of compounds changes in the same direction as temperature and time packaging.

CONCLUSION

The quantitative analysis of antioxidants, plasticizers and UV stabilizers by HPLC allowed to determine the concentrations of additives that migrated from plastic bags to *attiéké*. From 40°C to 70°C, migration is much more marked at the contact surface between the *attiéké* ball and the plastic bag. And this migration evolves in the same way as the temperature and conditioning time. At 30°C, migration is almost zero for all substances analyzed. From the results, it is clear that pollutants migrate from plastic bags to *attiéké* and this migration is much more pronounced when the temperature is high. Until an alternative to the current plastic bags is found, to limit the migration of additives and to protect the population from health risks, *attiéké* producers should pack this product at temperatures below 40°C and consumers should remove the surface layer.

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