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Enumeration and Identification of Main Fungal Isolates and Evaluation of Fermentation's Degree of Ivorian Raw Cocoa Beans

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Abstract: In Côte d'Ivoire cocoa production was dominated by smallholders and since the liberalization of the cocoa producing chain in 1999 Ivorian raw cocoa bean quality did not cease to lower and the causes are unspecified. The quality of raw cocoa beans is widely dependent on processing technologies for preventing the formation of unfermented or partly fermented beans and the development of fungi during storage. The target of this work was to evaluate the fermentation's degree of Ivorian raw cocoa and to compare the level of mould contamination and current fungal profiles of raw cocoa collected from three producing regions. A total of 90 raw cocoa beans samples was purchased and was analysed for the fermentation degree, enumeration and identification of fungi isolated. Moisture content and percentages of mouldy beans reached maximum values in black and clustered beans samples. Differences in each mould counts of cocoa beans in accordance with their physical appearance have been detected between cocoa producing areas selected. Black beans and clustered samples were infested by most fungi belonging to *Aspergillus* genus. Samples of both beans have been considered as poorer quality raw cocoa than brown beans samples. Six species of moulds belonging to four genera were isolated from all cocoa samples tested i.e. *Absidia corymbifera, Rhizopus oryzea, Aspergillus tubingensis, A. tamarii, A. llavus, Penicillium chrysogenum*. Early all species of fungi recovered in the present work can be considered as storage fungi. All cocoa samples collected from Alépé were contaminated by all identified fungal isolates. Fungal species belonging to *Aspergillus* genus were recorded in more of cocoa samples from Alépé than Soubré and Duékoué demonstrating that raw cocoa was poorly stored at Alépé.

Keywords: Fermentation's degree, enumeration, identification, fungi, quality, raw cocoa beans

INTRODUCTION

Cocoa is a very important ingredient in several kinds of foods, such as cakes, biscuits, child-foods, ice-creams and sweet consumed in developed countries. West Africa produces two-thirds of the World's cocoa (Anon, 2004). Côte d'Ivoire is the world's leading exporter of cocoa beans. Nowadays, one of the most widespread problems in advanced technological countries is food quality and safety. And raw cocoa bean and cocoa products quality is more and more in the heart of the future standards of quality of UE countries dealing with the presence of mycotoxins such ochratoxin A (EEC, 2005). In the same time, the economy of the most developing countries and particularly Côte d'Ivoire based primarily on their agricultural resources is strongly dependent on the standards and the often rigorous and rigid quality standards fixed by the developed countries. In Côte d'Ivoire cocoa production was dominated by smallholders and since the liberalization of the cocoa producing chain in 1999 Ivorian raw cocoa bean quality did not cease to lower and the causes are unspecified (DGTCP, 2004). The quality of cocoa beans is highly dependent on processing technologies and storage conditions for preventing the defective quality. Fermentation and drying are particularly important since they are largely responsible for the typical cocoa flavour precursors which develop later during the roasting of the beans and for the keeping quality of the raw beans (Niles, 1981). Generally, these processes are subject to

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local variations. For example fermentation may be carried out in baskets, heaps or boxes and may last from 36 hr to 6 days and drying may be done naturally in the sun and last from 7-8 days to 10-12 days depending on the harvest periods or season (Legrand, 1999). In quality control applications, cut test based on the colour changes in cotyledons during fermentation has been considered as good test (Shamsuddin and Dimmick, 1986) when determining the degree of cocoa beans fermentation, along with the formation of brown colour (Pettipher 1986; Misnawi *et al.,* 2003).

In all warm and wet countries, weather and agronomic conditions are favourable for fungi growth and Consequently food quality deterioration. In addition, storage and processing conditions of raw cocoa in the producing countries are not very safe and mycotoxinogenic fungi contamination may be possible at many critical points of the producing chain. As common agricultural food commodities, raw cocoa beans quality after harvest is influenced by a wide variety of abiotic and biotic factors. Mould contamination of food and feed is difficult to predict because it depends on a complex interaction of factors, such a temperature, moisture, endogenous fungal species, storage history and storage time (Chelack *et al.,* 1991). Generally, poor post-harvest management can lead to rapid deterioration quality by the initiation of fungal activity, to severely decreasing of commercial and nutritional values and to significant economic losses in foods and feeds (Magnoli *et al.,* 2006). Drying limits mould growth during transportation and storage, reducing raw cocoa bean moisture content from 60 to 8%. Exposure of raw cocoa to high moisture levels is most likely to occur at stages between post harvest and final consumption. Inefficient drying systems can also lead to fungal activity. Tournas and Katsoudas (2005) assumed that fungal spoilage of crops will depend on cultivation, harvesting, handling, transport, and post-harvest storage and marketing conditions. Fungal activity can result in contamination with mycotoxins and could pose a health risk for the consumers (Stinson *et al.,* 1981; Naresh *et al.,* 2003). In Côte d'Ivoire there is no available information on the natural occurrence of moulds in raw cocoa beans. So it is important to identify fungal contaminants in raw cocoa beans because some moulds can grow and produce mycotoxins on these commodities while certain moulds can cause infections or allergies. The main aims of this work were to enumerate and identify the main fungal isolates in Ivorian cocoa in order to carry out toxigenic and pathogenic strains after evaluation the beans fermentation's degree using cut test score. To the best of our knowledge, this is the first study of its kind in Côte d'Ivoire.

MATERIALS AND METHODS

Cocoa-sampling Regions:

Three cocoa producing regions in Côte d'Ivoire characterized by differing climatic conditions during harvesting and raw cocoa beans yield were selected for study. The regions were as follows: (i) Alépé, a southern area near Abidjan, a moderate hot rainy region with an average of 28-29°C during the harvest season, a low altitude, below 500 m, 70-80 mm/month rainfall and moderate cocoa yield; (ii) Duékoué a western area, a relatively cold, high rainy region with an average of 23-24°C and 70 mm/month rainfall, with relatively high altitude of 800-1000 m and relatively high cocoa produce, (iii) Soubré a western center region, with temperate, relatively high rainy region with an average of 25-28°C and 65-70 mm/month rainfall, with moderate altitude below 600 m and very high cocoa yield. Three representative farms (one farm per producing region) were selected for study.

Sampling:

During March-April of 2007 (towards the end of main harvest period), a total of 90 raw cocoa beans (*Theobroma cacao* L.) samples belonging to three types (brown, black and clustered) of beans was collected from the selected farms at the end of drying stage or during of storage. Ten samples (approximately 1 kg) from each type of beans and from each farm were taken at a single sampling time and kept inside in polythene bags during transport from the farm to the laboratory. The period from sampling to mycological analysis did not exceed a week. The farmers were questioned on processing history and storage time of their products.

Cocoa Bean Quality Analysis:

Moisture Content:

Moisture in the beans was determined using the International Organisation for Standardization (ISO) method (Hamid and Lopez, 2000). Approximately 10 g of ground bean sample was placed into a pre-weighed dish (W_1) with a lid and re-weighed to the nearest mg (W_2). The dish and contents were put in an oven at 103±2°C for 16 h. The lidded dish was then removed from the oven and put in desiccators to cool. After cooling they were weighed ((W_3) and the percentage moisture content calculated using the following formula:

$$Moisture\ content\ (\%) = \frac{(W_2 - W_3)\times 100}{(W_2 - W_1)}$$

Cut test score (%):

The cut test for sanitary and fermentation quality was performed according to the International method described by Hamid and Lopez (2000). A random sample of 300 cocoa beans was constituted and all beans were cut lengthwise using a sharp knife to expose a maximum cotyledon surface. Both halves of the cotyledon were examined visually in day-lighting. Observations were made for insect damage, mould infestation, germination as well as of the colour of the beans (slate, fully purple and fully brown). Slaty beans characteristics; include rubbery cotyledon, blackish colour, and resistance to cutting. Purple beans are produced when the fermentation has been terminated sooner than necessary. Fully brown beans may be well fermented beans. The results were expressed as a percentage. All analyses were done triplicate.

Mycological Analysis of the Cocoa Beans:

Subsamples of cocoa beans were stirred for 5 min at room temperature in 0.1% peptone water (1:10 w/v) and 0.1 mL was spread-plated onto Dichlroran Rose Bengal Chloramphenicol agar (DRBC) (Samson, 1991). The results (N) were expressed in $CFU.g^{-1}$ using the following formula (AFNOR 2002):

$$N = \frac{\sum C}{V\times(n_1 + 0.1\times n_2)\times d}$$

Where:

- $\sum^C$ is the sum of colonies count on all retained successive dilution Petri dishes;
- V is the volume of inoculums spread-plated onto DRBC;
- n_1 is the number of Petri dishes retained to the first dilution;
- n_2 is the number of Petri dishes retained to the second dilution;
- d is the dilution rate of the first retained dilution.
- Calculations were made with 95% confidence. The confidence interval (δ) characterizing the microbial dispersion in cocoa sample is calculated using as follows formula:

$$\delta = \left[\frac{\sum C}{B} \pm 1.96 \times \frac{\sqrt{\sum C}}{B}\right]\times\frac{1}{d}$$

With $B = V(n_1 + 0,1n_2)$

The moulds detected were isolated and subcultured on Czapeck-Dox agar for identification purposes. Predominant moulds were identified according to the identification key for common food-born fungi (Pitt, 1985; Pitt and Hocking, 1997). Fungal isolates were sent to BCCM™/MULC Culture Collection (Leuven Catholic University, B-1348 Louvain-la-Neuve, Belgium) for to complete or to confirm the identity.

Statistical Analysis:

Data were subjected to one-way analysis of variance (ANOVA) using JMP software Version 5 (SAS Institute, 2002) and significant differences between means were performed by Fisher Protected l.s.d. Test at P=0.05.

RESULTS AND DISCUSSIONS

Measurement of Cocoa Beans Fermentation Degree in Accordance with the Physical Appearance:

Table 1 presents the results of measurement of fermentation degree of 30 samples of each type of cocoa beans collected from Côte d'Ivoire. Moisture content of beans ranged around 8.43-8.87% except for clustered beans where the moisture content was 10.23%. Brown beans and clustered beans presented percentage of fully purple over 4%. Percentage of mouldy beans was particularly higher (12.79%) in black beans than in clustered

Table 1: Cut-test scores (%) of total cocoa beans samples in accordance with beans physical appearance (mean ± s.d., n=30)

Cocoa beans physical appearance	Quality/Characters						
	Average Moisture content	*Slaty*	*Fully purple*	*Brown*	*Germinated*	*Mouldy*	*Insect infested*
Brown beans	8.43±0.25[a]	1.83±0.74[ac]	4.44±1.28[a]	79,28±6.33[b]	1.28±1.20[a]	1.16±1.12[a]	0.84±0.68[a]
Black beans	8.87±0.18[a, c]	1.65±0.89[a]	3.15±1.36[a]	70,35±5.45[b]	2.41±2.41[a]	12.79±6.46[cd]	1.74±1.65[a]
Clustered beans	10.23±0.32[bd]	2.58±1.04[bc]	4.93±1.95[b]	51,11±3.77[a]	1.51±1.51[a]	7.63±3.16[bd]	1.01±0.70[a]

Different letters indicate significant differences at Fisher Protected l.s.d. test 5%

beans (7.63%). Brown beans showed the lowest percentage of mouldy beans (1.16%). Particularly low percentage of insect infested beans was recorded in all of the cocoa samples tested. The percentage of brown beans was above 70% in all samples except in clustered beans where it was very low around 50%.

Enumeration of Identified Main Fungi in Cocoa Beans Samples Tested:

Table 2 shows the mould counts in beans collected in according the physical appearance from three cocoa producing areas. Mould counts reached maximum values of 2.07×10^7 CFU.g^{-1} in black beans samples. Brown beans samples were lower contaminated (3.47×10^3 CFU.g^{-1}) than clustered beans (1.50×10^6 CFU.g^{-1}). A total of six species belonging to 4 genera was isolated from all cocoa samples analysed: *Aspergillus, Absidia, Rhizopus* and *Penicillium*. From the genus *Aspergillus* 3 species were isolated.

Table 2: Total main fungal isolates count (CFU.g^{-1}) [a] on DRBC agar in Ivorian raw cocoa samples collected in accordance with the beans physical appearance. (n=30 samples)

Main strains isolated	Mould count[b] (CFU.g^{-1})		
	Brown beans	Black beans	Clustered beans
Absidia corymbifera	1.3×10^3	2.0×10^6	5.50×10^4
Rhizopus oryzea	<50[c]	2.89×10^4	<50[c]
Aspergillus flavus	8.80×10^2	1.01×10^7	4.65×10^4
A. tubingensis	1.10×10^3	5.2×10^6	6.90×10^5
A. tamarii	1.51×10^2	1.40×10^6	2.02×10^5
Penicillium chrysogenum	6.88×10^1	2.1×10^6	9.80×10^3
Total mould count	3.47×10^3	2.07×10^7	1.50×10^6

[a] Spread plated
[b] Mean of thirty sampling of each beans quality
[c] Limit of method

The predominate strains isolated from the black beans samples were in decreasing order of importance *Aspergillus flavus* (1.01×10^7 CFU.g^{-1}), *A. tubingensis*, *Penicillium chrysogenum*, *Absidia corymbifera Rhizopus oryzea A. tamarii* with counts ranged from 1.40 to 5.2×10^6 CFU.g^{-1}. A few highly quantitative fungal species such as *Aspergillus tubingensis, A. tamarii*, *Absidia corymbifera, A. flavus* were recorded in clustered beans samples with counts values of 6.90×10^5, 2.02×10^5, 5.50×10^4 and 4.65×10^4 CFU.g^{-1}, respectively; while the same predominate species were found in brown beans but with the lowest counts values ranged from <50 to 1.3×10^3.UFC.g^{-1}.

Enumeration of Identified Fungal Isolates in Selected Cocoa Producing Areas:

Table 3 presents the main identified fungal species counts in all cocoa samples collected in each selected cocoa production area. Mould counts reached higher values of 1.87×10^7 CFU.g^{-1} in Alépé than Duékoué (2.01×10^6 CFU.g^{-1}) and Soubre (1.46×10^6 CFU.g^{-1}). All cocoa samples collected from Alépé were contaminated by all main identified fungal species while *Rhizopus oryzea* and *Aspergillus flavus* were not recorded in cocoa samples collected from Soubré and only *Rhizopus oryzea* was not isolated in those collected from Duékoué.

Rate of Cocoa Samples Contaminated by Each Identified Fungal Species in Accordance with Beans Physical Appearance:

Table 4 shows the rate of cocoa samples infected by each fungal species. Most of black cocoa beans samples were contaminated by *Aspergillus tubingensis* (79.17%) and *Absidia corymbifera* (70.83%) while 79.17 and 62.5% of clustered beans samples were contaminated by *Aspergillus flavus* and *Absidia corymbifer,* respectively. Although brown beans samples were relatively lower infected by isolated strains compared to others beans samples, the most frequently isolated strain in 33% of brown beans samples was *Absidia corymbifera*.

Table 3: Total main fungal isolates count (CFU.g^{-1})[a] on DRBC agar in cocoa beans in accordance with cocoa producing areas selected [n=30 samples (10 brown beans samples, 10 black beans samples, 10 clustered beans samples)]

Main strains isolated	Mould count[b] (CFU.g^{-1})		
	SOUBRE	ALEPE	DUEKOUE
Absidia corymbifera	6.19×10^5	1.10×10^6	7.75×10^5
Rhizopus oryzea	<50[c]	2.89×10^4	<50[c]
Aspergillus flavus	<50[c]	1.00×10^6	6.88×10^4
A. tubingensis	4.05×10^5	4.6×10^6	8.65×10^5
A. tamarii	4.08×10^5	9.00×10^5	2.68×10^5
Penicillium chrysogenum	2.68×10^4	2.00×10^6	2.96×10^4
et al.,Total mould count	1.46×10^6	1.87×10^7	2.01×10^6

[a] Spread plated

[b] Mean of thirty sampling collected from each area cocoa production

[c] Limit of method

Table 4: Rate of cocoa samples contaminated by each identified fungal species in accordance with beans physical characters (n= 30 samples)

Main strains isolated	% occurrence		
	Brown beans	Black beans	Clustered beans
Absidia corymbifera	33.33	70.83	62.5
Rhizopus oryzea	nd	8.33	nd
Aspergillus flavus	12.5	29.17	16.67
A. tubingensis	16.67	79.17	79.17
A. tamarii	4.17	45.83	33.33
Penicillium chrysogenum	4.17	16.67	12,5

Rate of Cocoa Samples Infected by Each Identified Fungal Species in Accordance with Cocoa Producing Area:

Table 5 shows the percentage of samples infected by each identified fungal isolates in accordance cocoa producing area. Most of cocoa samples collected from Alépé were contaminated by fungal species belonging to *Aspergillus* genus such as *Aspergillus tubingensis* (75%), *A. flavus* (58.33%) and *A. tamarii* (33.33%). Most of cocoa samples collected from Soubré and Duékoué were infected by *Absidia corymbifera* while *Rhizopus oryzea* was not recorded in any sample.

Table 5: Rate of cocoa samples contaminated by each identified fungal species in accordance cocoa producing area (n= 30 samples)

Main strains isolated	% occurrence		
	SOUBRE	ALEPE	DUEKOUE
Absidia corymbifera	66.67	37.5	62.5
Rhizopus oryzea	nd	8.33	nd
Aspergillus flavus	nd	58.33	4.17
A. tubingensis	50	75	50
A. tamarii	12.5	33.33	37.5
Penicillium chrysogenum	8.33	16.67	8.33

Discussion:

The cut test score of cocoa samples tested showed higher percentage of fully purple and lower percentage of brown beans in clustered beans than others beans samples. Beans clustering could have been caused either by harvesting of unripe pods or by poor separation of the beans and placentas during cocoa pods opening. So beans would be remained clustered with placentas during fermentation. The high percentage of purple beans indicates that a few beans have been properly fermented because biochemical and enzymatic reaction would not reach the inner beans in agglomerate mass. According to Bonvehi and Coll (1997) polyphenolic compounds are subjected to biochemical modification through polymerization and complexation with proteins conducting to the decreasing of poliphenols content (Wollgast and Anklam, 2000). At the same time anthocyanins are hydrolysed to anthocynanidins (Misanwi *et al.,* 2003) and disappear rapidly during the fermentation process, e.g. 93% were reportedly lost after 4 days fermentation (Wollgast and Anklam, 2000). The decreasing of polyphenols and anthocyanins content normally lead to the changes of cocoa beans colour from purple to brown. The purple beans contained high polyphenols and anthocyanins content after fermentation. That largely demonstrated and explained low percentage of brown beans.

Measurement of moisture content of cocoa samples showed that the mean moisture content in Ivorian raw cocoa beans was above critical value (8%) and clustered beans samples contained highest moisture content. The values agreed with those obtained in similar study (Legrand, 1999). The high moisture content in Ivorian cocoa was due probably to insufficient drying or inefficient drying systems. Indeed the growers did not take any more time to dry their product until the critical value of 8% because of hard competition between the exporters of cocoa. Indeed since the liberalization of the cocoa producing chain some exporters did not inspect the beans for moisture content and often preferred to buy the wet cocoa (moisture content above 8%) for fear it would be sold with the competitors. As they want to get money quickly the farmers would not pay too much attention to the moisture content of cocoa beans sold. In the others respects, the high moisture content recorded in cocoa samples collected could be also explained by the relatively high rainfall (average 60-65mm per month) in the selected areas during the cocoa sampling period. According to Hamid and Lopez (2000) the beans exchange moisture in accordance with the site. Therefore, properly dried cocoa beans could absorb moisture during the storage. This would imply a gain in moisture for raw cocoa.

The high moisture content in clustered beans is particularly due to the agglomeration of several beans. Beans clustering could have been caused either by harvesting of unripe pods, or by poor separation of the beans and placentas during cocoa pods opening. So beans would be remained clustered with placentas during and after fermentation. Therefore, during the drying stage some of clustered beans were not dried properly as previously demonstrated by Barel (1998). Indeed, the hot air in contact with external beans dries residual pulp and causes the formation of external and internal crusts. The presence of crusts slows down the departure of most of water from inner beans. At the end of drying stage, the inner beans contained largely higher moisture contents than those of external beans. Consequently, the whole clustered beans showed moisture content largely above critical moisture content (8%) for mould growth (Hansen, 1975).

Differences in percentage of mouldy beans have been detected between in all cocoa samples analysed. High percentage of mouldy beans in clustered and black beans samples could be favoured by their high moisture content as stated by Magan and Lacey (1988). According to Hamid and Lopez (2000) at moisture content above 8 per cent the cocoa beans are liable to mould development. High mouldy beans percentage in black beans are not surprising as regards the fact that these beans would be harvested from rotten pods as reportedly by Renaud (1954) or from pods which were stored during relatively long time before opening (Barel, 1998). These results agreed with those obtained by Renaud (1954) who reported that such beans are very liable to mould development during fermentation and drying stages. It must also be remembered that poor post-harvest management can lead to rapid invasion of stored agricultural commodities by moulds (Lacey and Magan, 1991). The moulds could penetrate the beans through the germ sections and spoil the cotyledons, the flavour and the whole bean (Crespo, 1986).

Difference in the number of the isolated strains was noted between the three cocoa beans types selected. The mould counts were generally higher in black and clustered beans samples than brown beans samples. Similar observations were made by several authors (Renaud, *et al.,* 1954; Guénot 1976, Oyeniran, 1980). As regards only the mould counts, black and clustered beans could be considered poor quality beans because according to Gourama and Bullerman (1995) high mould counts (i.e 10^6 UFC/g or higher) are generally thought to indicate poor quality grain with the possibility of the presence of mycotoxins. Fungal contaminants are also responsible for substantial effects in stored foodstuffs including discolouration, contribute to losses in nutritional value and to produce off-odours, deterioration in technological quality and contamination with mycotoxins (Basílico *et al.,* 2001, Magnoli *et al.,* 2006). Several authors have previously demonstrated that increasing of free fatty acids content in raw cocoa beans is mainly due to the action of storage fungi (Hansen *et al.,* 1973; Hansen 1975; Guenot *et al.,* 1976; Guéhi, 2003).

The lower mould counts in brown beans samples was probably due to the fact these beans are smooth, hard skin, therefore, impermeable to most fungi. As regards their lower mould contamination level than black and clustered beans, brown beans can be consequently considered as good quality cocoa.

The mycoflora is mainly represented by four genera in all the three different producing areas investigated. The identified species were similar demonstrating that the contamination came mainly from the air rather than the raw cocoa beans. Six species of moulds were isolated from cocoa collected i.e. *Absidia corymbifera, Rhizopus oryzea, Aspergillus tubingensis, A. tamarii, A. flavus, Penicillium chrysogenum*. All fungi species identified had already been recorded in cocoa in similar studies (Hansen, 1975; Guénot *et al.,* 1976; Bopaiah, 1992; Niles, 1981, Dharmaputra *et al.,* 1999). Nearly all species of fungi recovered in the present work can be considered common saprophytic soil organisms or storage fungi. The predominated fungi in black and clustered beans belonged to *Aspergillus* genus. The presence of *A. flavus* implies a risk of aflatoxin production even if no mycotoxin synthesis in Ivorian cocoa is revealed until today. Some fungi can start the spoilage from the field while others, although they could contaminate the pods in the field, actually proliferate and cause substantial spoilage only after harvest when the main plant defences are reduced or eliminated.

Differences in mould counts of cocoa beans have been also detected between cocoa producing areas investigated. Cocoa samples collected from Alépé are more spoiled than cocoa samples collected from Duékoué and Soubré. The results suggest that meteorological differences between cocoa growing areas are responsible for differences in moulds contamination level. The higher number in the south zone (Alépé) is probably due to the stagnant humidity which was always more than 70% because of it nearness of the sea. The second reason is probably related to the poorer post harvest management than other zones (Soubré and Duékoué). Indeed, nowdays at Alépé the cocoa plantations progressively give away to hevea culture. Consequently raw cocoa could be stored for a long time at the farmer's level under inappropriate conditions before the exporters' passage. The predominantly presence of several species belonging to *Aspergillus*'s genus and *Penicillium chrysogenum* in cocoa samples collected from Alépé demonstrated that raw cocoa had been invaded by moulds during storage. Indeed *Aspergillus* and *Penicillium* spp. are generally considered by Ramakrishna *et al.* (1996) as typical storage species.

The rates of black and clustered beans samples contaminated by several species belonged to *Aspergillus* genus and *Absidia corymbifera* were more 70%. Data are similar with the ones of Niles (1981) and Dharmaputra *et al.* (1999). No clustered and brown beans sample was contaminated by *Rhyzopus oryzea* while its frequency in black beans samples is the lowest. *Penicillium chrysogenum* have also contaminated the lower percentage of cocoa samples whatever their physical appearance. The presence of both strains (*R. oryzea* and *P. chrysogenum*) can be considered as accidental or occasionally contamination. The lower percentage of brown beans samples invaded by each identified species than black and clustered beans confirms their better quality. The lowest percentage of contaminated samples by any isolate were recorded at Soubré and Duékoué while all identified fungi were isolated in the most of cocoa samples collected from Alépé. *R. oryzea* and *A. flavus* were occasionally isolated from a few cocoa samples collected from Soubré and Duékoué. Both fungi could be considered as occasionally strains in these cocoa producing areas. This data is not agreement with Niles (1981) and Dharmaputra *et al.* (1999) which considered *A. flavus* as the dominant mould flora in the raw cocoa. Although cocoa samples collected form Soubré contained highest moisture content, they were lower contaminated by storage fungi than those properly dried demonstrating that the contamination of cocoa beans was not linked only to the moisture content. Indeed according to Naresh *et al.* (2003) crops quality after harvest is influenced by a wide variety of abiotic and biotic factors. The moisture content and temperature are effectively the most important variables in determining growth of fungi (Magan and Lacey, 1988) but generally mould contamination of food is difficult to explain because it depends on a complex interaction of factors, such a temperature, moisture, kind of food, endogenous fungal species, storage history and storage time (Chelack 1991).

All the isolated mould strains could not synthesise mycotoxin except *A. flavus* which are dominant only in cocoa collected from Alépé but until today, no mycotoxin production was revealed in Ivorian cocoa. Additionally, as most of raw cocoa in Côte d'Ivoire is mainly originated from Soubré and western zones (Duékoué) we can consider Ivorian cocoa as relatively good quality which needs to be improved considering fermentation process and storage conditions.

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