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Full Length Research Paper

A study of fungal profiles of cocoa produced in Côte d'Ivoire in order to discover whether toxigenic and pathogenic moulds are present

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The degree of fermentation and incidence of contaminating fungi were determined in 90 raw cocoa bean samples. Excessive moisture content and low percentages of brown beans are recorded in cocoa samples from Soubré and Alépé, corresponding to maximum value of percentages of purple, mouldy and insect infested beans. Differences in mould counts were detected in cocoa beans according to the producing regions; cocoa from Alépé was most contaminated by moulds. Six fungi were commonly isolated that is, *Absidia corymbifera, Rhizopus oryzae, Aspergillus tubingensis, Aspergillus tamarii, Aspergillus flavus and Penicillium chrysogenum.* Nearly all of the fungi recovered can be considered as storage fungi. Studies of cocoa samples revealed that raw cocoa beans collected from Duékoué appeared to be of better quality than that from Alépé and Soubré.

Key words: Cocoa, fermentation, fungi, quality.

INTRODUCTION

Nowadays, one of the most widespread concerns in advanced technological countries is food quality and safety. The economy of most developing countries, based primarily on their agricultural resources, is strongly dependent on the often rigorous and rigid quality standards set by developed countries. Cocoa is a very important ingredient in several kinds of foods, such as cakes, biscuits, child-foods, ice-creams and sweets. Cocoa beans are an important source of cocoa powder and food worldwide and constitute an inexpensive fat source and are the principal raw material of chocolate from Africa and both Central and South America (Tafuri et al., 2004). Export of raw cocoa beans is of great economic importance in several tropical countries. West Africa produces two-thirds of the World's cocoa, with Côte d'Ivoire and Ghana as the major producers (Anon, 2004). Cocoa beans are seeds from fruit pods of the tree *Theobroma cacao* L. Each pod contains 30 - 40 beans, embedded in a mucilaginous pulp. Following opening of the pods, cocoa beans become contaminated naturally with a variety of micro-organisms, originating from workers' hands, containers for transport, knives, pod sur-faces, etc. Fresh cocoa beans have an astringent, unpleasant taste. Such product has to be fermented, dried and roasted in order to obtain raw cocoa beans with desired characteristic flavour and taste (Thompson et al., 2001) . Beans are piled in either heaps, boxes, trays or small baskets, covered with plantain leaves and left to ferment for 5 - 7 days (Fowler, 1999). During cocoa bean fermentation, sugars in the mucilage are converted into alcohol by yeasts which proliferate after the sterile mass from the pods is exposed to the surrounding air (Ardhana and Fleet, 2003) . The mass then becomes runny and drops away from the beans. An enzymatic reaction takes place killing the embryo and contributing to the formation of flavour and the colour changes in cotyledons from purple to brown.

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Fermentation reactions have been reviewed by Fowler (1999) and Beckett (2000) . At the end of cocoa fermentation, changes in colour are widely used to predict flavour potential of cocoa beans. During the quality control, a cut test to observe changes in cotyledon colour during fermentation has been considered as a good indicator (Shamsuddin and Dimmick, 1986) when determining the degree of fermentation of cocoa beans (Pettipher, 1986; Misnawi et al., 2003). Thereafter, drying to reduce moisture content of beans from 60 to 8% limits mould growth during transportation and storage. As is common with agricultural food commodities, raw cocoa bean 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 as temperature, moisture, endogenous fungal species, storage history and storage time (Chelack et al., 1991). Important factors include processing technologies and storage conditions for preventing the development of mould (Magan et al., 2003). Exposure of cocoa beans to high humidity is most likely to occur at stages between harvest and final consumption. Then, poor post-harvest management can lead to rapid deterioration in the quality of cocoa, severely decreasing of commercial and nutritional values. Fungal activity can also cause undesirable effects in food commodities including discolouration and deterioration in quality, and can result in contamination with mycotoxins (Magan et al., 2003). It is important to note that Ivorian raw cocoa bean quality did not escape being degraded since the liberalization of the cocoa-producing chain in 1999 and the reasons were unspecified (DGTCP, 2004). Therefore it is important to identify the factors that reduce commercial value by studying the degree of fermentation and incidence of contaminating fungi in some samples of Ivorian cocoa beans because moulds can produce mycotoxins. The objective of this study did not focus on post-harvest handling and technology processing of cocoa beans at the farmer's level in Côte d'Ivoire.

First, we purchased raw cocoa beans from three main Ivorian producing regions in order to compare their degree of fermentation. Secondly, the study explored the fungal profiles of cocoa produced in Côte d'Ivoire in order to discover whether toxigenic and pathogenic moulds are present.

MATERIALS AND METHODS

Cocoa-producing regions

Three cocoa producing regions in Côte d'Ivoire, characterized by differing climatic conditions during harvesting and raw cocoa bean 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.

Sampling

During the first 3 months of the 2007 harvest period, 90 raw cocoa bean samples belonging to three types (brown, black and clustered) of bean was collected from three farms in each of three selected regions at the end of the drying stage or during storage. Currently when cocoa beans processing technologies were carefully conducted both cocoa beans surface and inside colour is brown. But in some case such as when a) cocoa beans were extracted from rotten or decayed pods, b) beans were dried in iron surface or c) they are get wet by the rains, their only surface's colour changed from brown in to black. Clustered beans came from fermentation of beans with the rachis together.

Thereafter drying of cocoa, the mass of beans were obtained and constituted clustered beans type. They are characterized by current high moisture content up to 8% and by several beans up to two in the same cluster. Ten samples (each approximately 1 kg) of each descriptive type of bean were taken from each farm 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 beans quality analyses

Moisture content

Moisture in the beans was determined using the International Organisation for Standards (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). Dishes and contents were put in an oven at 103 ± 2°C for 16 h. After cooling in desiccators they were weighed (W_3) and the percentage moisture content was calculated using the following formula:

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

Cut test score (%)

The cut test for sanitary and fermentation quality of beans was performed according to the International method described by Hamid and Lopez (2000). Random samples of 300 beans for each cocoa sample were cut lengthwise using a sharp knife to expose a maximum cotyledon surface. Both halves of the cotyledon were examined visually in daylight. Observations were made for insect damage, mould infestation, germination as well as of the colour of the beans (slate, fully purple and fully brown). Slate bean characteristics include rubbery cotyledon, blackish colour, and resistance to cutting. Purple beans occur when the fermentation has been terminated prematurely. Fully brown beans are well-fermented beans. Results were expressed as a percentage and all analyses were done triplicate.

Mycological analysis of the cocoa beans Sub-samples of cocoa beans were stirred for 5 min at room tempe-

Table 1. Cut test scores (%) of each type of 10 cocoa beans samples collected from Alépé. Values are means of 10 samples of each type of beans. Within columns, values followed by the same letters are not significantly (P = 0.05) different according to the Fisher Protected l.s.d. test.

Types of cocoa beans samples	Quality/Characters							
	Average Moisture content	Slaty	Fully purple	Purple brown	Brown	Germinated	Mouldy	Insect infested
Brown beans	6.72±0.60^{a}	1.52±0.69^{bd}	1.71±0.58^{ac}	0.77±0.12^{a}	87.19±3.88^{bd}	2.74±0.87^{a}	2.31±0.92^{a}	1.83±0.54^{a}
Black beans	7.94±0.69^{ac}	0.81±0.44^{a}	1.64±0.71^{a}	0.79±0.19^{ac}	62.45±4.15^{a}	4.57±1.38^{cd}	20.62±2.66^{ce}	4.00±0.67^{bd}
Clustered beans	9.58±0.30^{bd}	2.43±1.24^{ce}	4.29±1.38^{bd}	2.12±0.61^{bd}	72.76±6.15^{ac}	3.57±0.49^{bd}	8.40±1.72^{bd}	2.33±0.85^{ad}

temperature in 0.1% peptone water (1:10 w/v) and triplicate 0.1 mL of each dilution was spread on three 90 cm Petri plates of Dichloran Rose Bengal Chloramphenicol agar (DRBC) (Samson, 1991). The results (N) were expressed in CFU.g^{-1} using the following formula (AFNOR, 2002):

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

Where:

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 are carried out with a probability of 95%. The confidence interval was calculated by the following formula:

$$\delta = \frac{C}{BBd} \pm 1{,}96\sqrt{C} \times 1$$

Where $B = V(n_1 + 0{,}1n_2)$

Moulds detected were isolated and sub cultured on Czapeck-Dox agar for identification purposes. All isolated moulds were identified firstly by the macroscopic characters and then according to the identification keys for common food-born fungi (Pitt, 1985; Pitt and Hocking, 1997). Fungal isolates were sent to BCCM^{TM}/MULC Culture Collection (Leuven Catholic University, B-1348 Louvain-la- Neuve, Belgium) for to complete or to confirm the identity of moulds.

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

Measurement of the degree of fermentation of cocoa beans

Results of cut test analysis of cocoa beans collected from Alépé are summarized in Table 1. They showed that clustered cocoa beans samples had high percentage of fully purple beans with 4.29 ± 1.38%. Black beans samples presented very high percentage above 20% of mouldy beans. Percentage of insect infested beans was low except for black beans type. Table 1 also showed that only clustered beans presented moisture content above 8%. Cocoa collected from Duékoué showed the same percentage of fully purple beans for all studied types of beans (Table 2). But black and clustered beans samples had higher percentages of mouldy beans of 12.32 ± 9.64% and of 9.43 ± 4.47% respectively than brown beans (0.84 ± 0.69%). Low percentage of insect infested beans was found in all cocoa samples from Duékoué whatever the quality (ranged from 0.33 to 2.14%). Clustered beans showed relatively high moisture content of 8.41 ± 1.28% and lower percentage of purple beans (3.88 ± 1.09%) than black beans.

Table 3 presents the results of measurement of moisture content and of the degree of fermen-tation of cocoa collected from Soubré. All tested samples showed moisture contents above 8% whatever their physical characteristics: 11.11, 11.44 and 12.69% in respectively brown, black and clustered beans. In others respects, clustered beans showed higher percentage of fully purple beans (7.10%) than all others beans. Highest percentage of mouldy beans were obtained only in black and clustered beans of 8.86 ± 2.96% and 5.17 ± 1.84% respectively. The percentage of insect infested beans is low whatever the physical quality of cocoa beans analysed (ranged from 0.27 to 2.60%) . Results of cut test scores of cocoa collected from each cocoa producing region are shown in Table 4. The highest moisture content (11.74%) is measured in cocoa from Soubré. Cocoa collected from Duékoué and Soubré

Table 2. Cut test scores (%) of each type of cocoa beans samples collected from Duékoué. Values are means of 10 samples of each type of beans. Within columns, values followed by the same letters are not significantly (P = 0.05) different according to the Fisher Protected l.s.d. test.

Types of cocoa beans samples	Quality/Characters							
	Average Moisture content	Slaty	Fully purple	Purple brown	Brown	Germinated	Mouldy	Insect infested
Brown beans	7.47±1.14^{ab}	2.07±1.01^{ab}	4.07±1.38^{ab}	1.88±0.53^{a}	82.60±3.21^{c}	0.74±0.68^{a}	0.84±0.69^{a}	0.33±0.24^{a}
Black beans	7.23±0.89^{a}	2.82±2.12^{b}	4.36±3.21^{b}	3.05±1.26^{b}	64.68±5.61^{a}	3.40±1.93^{c}	12.32±9.64^{c}	2.14±0.76^{c}
Clustered beans	8.41±1.28^{b}	2.02±1.09^{a}	3.88±1.09^{a}	4.53±2.31^{b}	70.18±4.19^{ab}	0.79±0.70^{ab}	9.43±4.47^{bc}	0.76±0.58^{ab}

Table 3. Cut test scores (%) of each type of cocoa beans samples collected from Soubré. Values are means of results of 10 samples of each type of beans. Within columns, values followed by the same letters are not significantly (P = 0.05) different according to the Fisher Protected l.s.d. Test.

Types of cocoa beans samples	Quality/Characters							
	Average Moisture content	Slaty	Fully purple	Purple brown	Brown	Germinated	Mouldy	Insect infested
Brown beans	11.11±0.92^{a}	1.98±0.36^{a}	8.26±3.42^{b}	4.61±0.47^{b}	78.96±3.21^{b}	0.52±0.46^{a}	0.29±0.22^{a}	0.27±0.22^{a}
Black beans	11.44±0.98^{a}	2.05±0.69^{a}	3.64±0.69^{a}	1.77±0.68^{a}	77.49±1.68^{b}	0.93±0.69^{b}	8.86±2.96^{c}	2.60±1.19^{b}
Clustered beans	12.69±0.79^{b}	3.33±0.90^{b}	7.10±2.10^{b}	8.68±1.27^{c}	71.65±2.13^{a}	0.43±0.36^{a}	5.17±1.84^{b}	0.56±0.53^{a}

showed highest percentage of fully purple beans. The highest percentage of mouldy beans was recorded in cocoa purchased from Alépé with 10.43 ± 8.00%. But the same percentage of slaty beans (1.56 to 2.41%) and the same level of brown beans (70%) were recorded in cocoa whatever the origin. In average, all cocoa samples studied presented 9.78% moisture content, 4.17% of fully purple beans, 7.18% of mouldy beans. Moulds counts and fungal profile in each type of beans.

Overall, levels of fungal contamination of tested cocoa samples purchased from each cocoa producing region are summarized in Tables 5. Mould counts in cocoa analysed reached maximum values of 541.98 ± 0.14×10^{5} and 186.16 ± 0.70×10^{4} CFU.g^{-1} in black beans and clustered beans respectively at Alépé. There was a quailtative uniform distribution of filamentous fungi in samples tested. At Duékoué, higher levels of mould counts (434.59 ± 0.55×10^{4} and 167.1 ± 0.60×10^{4} CFU.g^{-1}) were recovered in black and clustered beans respectively than brown beans (2.60 ± 1.35.10^{3} CFU.g^{-1}). Six strains of filament-tous fungi such as *Absidia corymbifera* (Cohn) Saccardo and Trotter, *Rhizopus oryzae* Went and Prinsen Geerlings, *Aspergillus tubingensis* (Scho-ber) Mosseray, *Aspergillus tamarii* Kita, *A. flavus* Link : Fries, *Penicillium chrysogenum* Thom, were found in black beans while a few fungi (*Asper-gillus tubingensis, Absidia corymbifera*) were recorded in brown beans from Duékoué. There was a qualitative variable distribution of filament-tous fungi in cocoa samples tested in accordance with their quality. Cocoa collected from Soubré showed most infected beans. Indeed black beans and clustered beans cont-ained 341.01 ± 0.35 × 10^{4} and 96.20 ± 0.554 × 10^{4} CFU.g^{-1} respectively. At this region, several strains of filamentous fungi were found in black beans samples while two strains were recorded in clustered beans and only one strain was isolated from brown beans samples.

DISCUSSION

In order to evaluate the commercial value of cocoa according to the type of beans 90 samples were collected from three Ivorian mains cocoa producing regions. The degree of fermentation and moisture content were measured. Moisture content of clustered beans is higher than tolerable limits (8%) whatever the producing area. That high moisture content is due to the agglomeration of several beans. Beans clustering could has been caused either by harvesting of unripe pods, or by poor separation of beans and placenta during cocoa pods opening. So beans would be remained clustered within the placenta during and after fermentation. Therefore, drying induces clustered beans which were not dried properly as previously demonstrated by Barel (1998). Indeed, the hot air in contact with external cocoa beans

Table 4. Cut test scores (%) of all cocoa beans samples collected from each producing region. Values are means of results of all 30 samples from each selected region. Within columns, values followed by the same letters are not significantly (P = 0.05) different according to the Fisher Protected l.s.d. test.

Cocoa producing regions	Quality/Characters							
	Average moisture content	Slaty	Fully purple	Purple brown	Brown	Germinated	Mouldy	Insect infested
Alépé	8.08±1.31^{a}	1.56±1.05^{a}	2.53±1.53^{a}	0.97±0.61^{a}	70,21±5.21^{a}	3.62±1.28^{c}	10.43±8.00^{b}	2.60±1.19^{c}
Duékoué	7.71±1.19^{a}	2.10±0.80^{b}	4.03±1.19^{b}	2.29±1.03^{b}	76,02±7.35^{b}	1.01±0.99^{a}	6.31±5.20^{a}	0.56±0.53^{a}
Soubré	11.74±1.11^{b}	2.41±0.90^{c}	5.96±2.87^{c}	3.09±1.11^{c}	71,23±6.21^{a}	0.56±0.54^{a}	4.79±3.97^{a}	0.22±0.27^{a}
Total samples	9.78±0.25^{a}	2.02±0.25^{b}	4.17±0.47^{b}	2.16±0.12^{b}	71,84±3.56^{a}	1.73±0.29^{b}	7.18±0.81^{a}	1.12±0.18^{b}

Table 5. Total mould count (CFU.g^{-1}) on DRBC agar and main fungal isolates in each type of cocoa beans collected from Alépé, Duékoué and Soubré. Values are means of results of 10 samples of each type of beans. Within columns of each cocoa producing region, values followed by the same letters are not significantly (P=0.05) different according to the Fisher Protected l.s.d. test.

Cocoa producing regions	Types of cocoa beans	Mould count (CFU.g^{-1})	Main isolates strains isolated (ranked in order of relative importance)
Alépé	Brown beans	6.27±2.05×10^{3a}	*Aspergillus tubingensis, A. flavus, Absidia corymbifera, Penicillium chrysogenum*
	Black beans	54198.37±13.82×10^{3c}	*Absidia corymbifera, Aspergillus tubingensis, A. tamarii, A. flavus, Penicillium chrysogenum*
	Clustered beans	1861.61±7.03×10^{3b}	*Absidia corymbifera, Aspergillus tamarii, A.tubingensis, Penicillium chrysogenum*
Duékoué	Brown beans	2.60±1.35×10^{3a}	*Aspergillus tubingensis, Absidia corymbifera*
	Black beans	4346.92±5.55×10^{3b}	*Absidia corymbifera, Rhizopus oryzae, Aspergillus tubingensis, A. tamarii, A. flavus, Penicillium chrysogenum*
	Clustered beans	1671.86±5.97×10^{3c}	*Absidia corymbifera, Aspergillus tamarii, A. tubingensis, Penicillium chrysogenum*
Soubré	Brown beans	1.53±0.82×10^{3a}	*Absidia corymbifera*
	Black beans	3410.14±3.53×10^{3c}	*Absidia corymbifera, Aspergillus tubingensis, A. tamarii, Penicillium chrysogenum*
	Clustered beans	961.95±5.44 ×10^{3b}	*Absidia corymbifera, Rhizopus oryzae*

would dry residual pulp and causes the formation of internal and external crusts. These crusts slow down the departure of most of water from internal beans. At the end of drying stage, the internal beans contain moisture contents largely higher than those of external beans. Consequently, whole clustered beans show moisture content higher than the critical moisture content (8%) for mould growth (Hansen, 1975). Highest moisture contents were recorded in cocoa beans collected from Soubré. These moisture contents could be due to insufficient or inadequate drying system of cocoa linked to two raisons. Firstly, according to the investigations that we have carried out, the growers do not take any more time to dry their product until the water content reached 8% because of hard competition between the exporters of cocoa since the liberalization of the cocoa chain. At farmer's level, some exporters did not inspect cocoa beans correctly for moisture content determination and often preferred to buy wet cocoa beans (moisture content above 8%) for fear it would be sold with the competitors. In the others respects, at Soubré farmers did not pay too much attention to the moisture content of cocoa beans sold, because they want to get money quickly. Secondly, the high moisture content observed in cocoa collected from Soubré could be due to the high rainfall (65 - 70 mm/month) which can increase the relative humidity in this region during the harvest. Indeed, 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. In others regions such as Alépé and Duékoué, cocoa's growers seemed to observe carefully official recommendations about the conduction of cocoa drying in order to obtain final moisture content around of 8%. Although the percentage of brown beans was above 60% in all samples collected, the percentage of fully purple beans was highest in clustered beans (4.29%) collected from Alépé; brown and clustered beans from Soubré in a discordance with beans appearance. According to Misnawi et al. (2003) high percentage of fully purple cocoa beans is not considered as a severe default of cocoa quality because some countries such as Malaysia

are currently making use of the unfermented and partly fermented cocoa beans especially for cocoa liquor, powder and cocoa butter production. But because of the excessive astringency and bitterness of such cocoa beans, fully purple beans are undesirable in West Africa where fermentation process is strongly recommended to be made during 5 - 7 days. This treatment aims to reduce considerably the content of polyphenols, to lead to the change of beans internal colour from purple to brown (Pettipher, 1986) and to confer to cocoa a good chocolate's flavour potential (Misnawi et al., 2003). Indeed, during cocoa fermentation polyphenols are subjected to biochemical modification through polymerization and complexation with protein, hence decreasing astringency (Bonvehi and Coll, 1997). At the same time anthocyanins are hydrolysed to anthocyanidins and usually disappear rapidly during the fermentation process, e.g. Wollgast and Anklam (2000) have reported a lost of 93% after 4 days fermentation. So at Duékoué, fermentation pro-cessing seems not properly conducted. According to the growers of this region, the fermentation process was stopped sooner (below 4 days) than necessary because of the thieves since the increasing of insecurity due to the war situation in Côte d'Ivoire since 2002.

Some cocoa farmers of region of Soubré recognize that they fermented their cocoa during short duration likely the growers at Duékoué because of the high yield of cocoa per week. High percentage of fully purple beans in clustered beans collected from Alépé could be due to the formation of clusters probably due to the absence of the manually daily turning of the fermentation mass. However, according to Crespo (1986) many Forastero beans often have a purplish tone even after proper fermentation. As any information about the genotypic origin of collected cocoa beans is unavailable, no conclusion can be made on the causes of formation of purple beans in samples studied. But on the basis of current results, the cocoa collected from Soubré is poorly fermented compared to the cocoa collected from Duékoué and Alépé.

Differences in mould counts of cocoa beans have been detected between cocoa producing regions. Higher percentages of mouldy beans were recorded in black and clustered beans samples whatever their origins than brown beans. But cocoa collected from Alépé is more contaminated by moulds than cocoa produced at Soubré and Duékoué. Differences in moulds contamination level seem to be linked to meteorological differences between cocoa growing. Surprising observation of an inverse relationship in Soubré between moisture content and mouldiness was made. This relationship could be linked to the fact cocoa beans were freshly prepared and then shortly stored at framers level when they we re collected. In the others respects, at this region cocoa beans were not properly dried before selling because of the abundance of the cocoa's production and the increasing competition between the multinational cocoa companies' traders.

Highest percentage of mouldy beans in black beans could be attributed to their probable origin from rotten pods or pods which were stored during long time before opening. These results agreed with those of Renaud (1954) who reported that such beans are easily contaminated by the moulds during fermentation and drying stages. They confirmed also that poor post-harvest management can lead to rapid invasion of stored agricultural commodities by moulds (Lacey and Magan, 1991). Moulds could penetrate the beans through the germ sections and spoil cotyledons, the flavour and the whole bean (Crespo, 1986). Mechanical damages are also conducive to entry of spoilage fungi in insufficiently dried grains. According to Bopaiah (1992) the presence of germinated and broken beans could also favour the formation of mouldy beans. Percentage of mouldy beans in clustered beans could be linked to their high moisture contents as stated by Magan and Lacey (1988) . Analysis of percentage of mouldy beans of each type of cocoa carried out that percentage of mouldy beans seems to be positively correlated with the percentage of insect infested beans. Those observations would confirm that some storage insects disseminated storage fungi as previously suggested by Sinha (1971). As cut test is usually adopted at the international market allowing the maximum mouldy beans of 3% for grade 1 and 4% for grade 2 respectively, only brown cocoa beans can consider as good quality cocoa. Highest mould count up to 4% was observed in black and clustered beans whereas brown beans showed good quality characteristics whatever the cocoa producing region. These observations are not surprising considering the fact that black beans could be obtained from rotten pods as reportedly by Renaud (1954) and clustered beans presented high moisture content (Hansen, 1975). Black and clustered beans could be again considered as poor quality beans because highest mould counts (that is, 10^6 UFC/g) with the risks of the production of mycotoxins (Gourama and Bullerman 1995). As brown beans are smooth, hard skins, they were impermeable to most fungi. Although mouldy beans were determined based on two different methods, namely cut test and plating methods on DRBC, results of mouldy beans seem to be positively correlated with mouldy beans. Cocoa collected from Alépé is most contaminated by moulds. This strong contamination could be due to the fact that culture of cocoa would have passed in the second plan to the profit of others crops such as hevea. Consequently ferment and dried cocoa beans could be remained probably in storage for a long time at the farmer's level under inappropriate conditions.

Six species of moulds were isolated from cocoa beans collected that is, *A.corymbifera, R. oryzae, A. tubingensis, A. tamarii, A. flavus, P. chrysogenum.* All fungi generaly identified had already been recorded in cocoa from different cocoa producing areas (Hansen, 1975; Guénot et al., 1976; Niles, 1981, Bopaiah, 1992; Dharmaputra et al., 1999). Nearly all species of fungi re-

covered in the present work can be considered as common saprophytic soil organisms or storage fungi. Post harvest moulds development in cocoa can be expected to relate to unfavourable climates for drying and storage: poor drying practice or quality control, inadequate or poor storage conditions. No ochratoxin A- producing fungi were isolated from any samples tested but unfortunately one potential aflatoxin producing fungus such as *A. flavus* was recorded in some samples from Alépé and Duékoué. As mycotoxin-producing abilities of fungi isolated from raw cocoa beans were not elucidated in this study, very high mould counts in black and clustered beans showed good evidence to support their potential toxigenic abilities and to extend this study to others cocoa producing regions in the East of Côte d'Ivoire for example.

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REFERENCES

AFNOR (2002). Microbiologie alimentaire. (Saint-Denis La Plaine Cedex).

Anon (2004). Annual report for 2001/02. International Cocoa Organization; https://www.aginternetwork.net/http://www.icco.org. (January 2005)

Ardhana M, Fleet GH (2003). The microbial ecology of cocoa fermentations in Indonesia. Int. J. Food Microbiol. 86:87-99.

Barel MA (1998). Première transformation du cacao. Formation de l'arôme cacao. In Pontillon J (eds) Cacao et chocolat. Production, utilisation et caractéristiques, Lavoisier Tec & Doc., Collection Sciences et Techniques Agroalimentaires, Paris, pp 96-115.

Beckett ST (2000). The Science of Chocolate. Royal Society of Chemistry Paperbacks.

Bonvehi JS, Coll FV (1997). Evaluation of purine alkaloids and diketopirazines contents in processed cocoa powder. Food Chemistry, 60:365-370.

Bopaiah BM (1992). Deterioration of processed cocoa beans in storage and mycotoxin. Indian Cocoa. Arecanut & Spices Journal, XVI:11-13.

Chelack WS, Borsa J, Marquardt R, Frohlich AA (1991). Role of the competitive microbial flora in the radiation-induced enhancement of ochratoxin production by *Aspergillus alutaceus* var. *alutaceus* NRRL 3174. Appl. Environ. Microbiol. 57:2492-2496.

Crespo S (1986). Cacao beans today. Pennsylvania, Lititz.

DGTCP (Direction Générale du Trésor et de la Comptabilité Publique de Cote d'Ivoire) (2004) Commercialisation du cacao: La Cote d'Ivoire s'apprête à relever le défi de la certification. Article 140 online www.tresor.gov.ci/actualités (may 2005)

Dharmaputra OS, Amad SM, Retnowati I, Wahyudi T (1999). The occurrence of insects and moulds in stored cocoa beans at South Sulawesi. Biotropia, 12:1-18.

Fowler MS (1999). Cocoa beans: From Tree to Factory. In Beckett ST (eds) Industrial Chocolate manufacture and use, Blackwell Science, Oxford, pp 137-152.

Gourama H, Bullerman LB (1995). Relationship between Aflatoxin Production and Mold Growth as Measured by Ergosterol and Plate Count. Lebensm.- Wiss. U-Technol. 28: 185-189.

Guénot M-C, Perriot J -J, Vincent J-C (1976). Evolution de la microflore et des acides gras des fèves de cacao au cours du stockage: étude préliminaire. Café, Cacao, Thé**,** 30:53-58.

Hamid A, Lopez AS (2000). Quality and weight changes in cocoa beans stored under two warehouses' conditions in East Malaysia. The planter, Kuala Lumpur 76:619-637.

Hansen AP (1975). Microbiological activity and its effect on cocoa beans. The Manufacuring Confectioner 55:35-39.

Lacey J, Magan N (1991). Fungi colonising cereal grain: Their occurrence and water and temperature relationships. In Chlkowski J (eds) Cereal grain – Mycotoxins, Fungi and Quality in Storage, Elsevier, Amsterdam, pp 77-118

Magan N, Lacey J (1988). The phylloplane microbial population of wheat and effect of late fungicide applications. Annals of Appl. Biol. 109:117-128.

Misnawi, Jinap S, Jamilah B, Nazamid S (2003). Effects of incubation and polyphenol oxydase, enrichment on colour, fermentation index, procyanydins and astringency of unfermented and partly fermented cocoa beans. Int. J. Food Sci. Technol. 38:285-295.

Magan N, Hope R, Cairns V, Aldred D (2003). Post-harvest fungal ecology: Impact of fungal growth and mycotoxin accumulation in stored grain. Eur. J. Plant Pathol. 109:723-730.

Niles EV (1981). Microflora of imported cocoa beans. J. of stored Products and Res. 17:147-150.

Pettipher GL (1986). An improved method for the extraction and quantification of anthocyanins in cocoa beans and its use as an index of the degree of the fermentation. J. Sci. Food Agr. 37:289-296.

Pitt JI (1985). Laboratory Guide to common Penicillium species CSRIO Division of Food Research. North Ryde, New South Waler, Australia.

Pitt JI, Hocking AD (1997). Fungi and Food spoilage. 2nd edn London, Blackie Academia and Professional.

Renaud R (1954). La qualité du cacao. Les moisissures des fèves fermentées. Agronomie tropicale (Nogent-sur-Marne) 9:563-583

Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O (1995). Introduction to Food-Borne Fungi. Delft Netherlands: Centraalbureau voor Schimmelcultures Baarn.

SAS Institute, Inc. (2002). JMP Software Version 5 (Cary, NC)

Shamsuddin SB, Dimmick PS (1986). Qualitative and quantitative measurement of cacao beans fermentation. In Pennsylvania State University (eds) Proceeding of the Symposium of Cacao Biotechnology, pp 55-78.

Sinha RN (1971). Fungus as food for some stored product insects. J. Econ. Entomol. 64:3-6

Tafuri A, Ferracane R, Ritieni A (2004). Ochratoxin A in Italian marketed cocoa products. Food chemistry**.** 88:487-494.

Thompson SS, Miller KB, Lopez AS (2001). Cocoa and coffee. In Doyle et al. (eds) Food Microbiology - Fundamentals and Frontiers, ASM Press, Washington, pp. 721-733.

Wollgast J, Anklam E, (2000). Review on polyphenols in *Theobroma cacao*: changes in composition during the manufacture of chocolate and methodology for identification and quantification. Food Res. Inter. 33:423-447.