# PREVALENCE OF *BACILLUS CEREUS* AND EMETIC STRAINS DETECTION FROM IVORY COAST LOCAL FLOURS

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## ABSTRACT

*Bacillus cereus* is a pathogen associated with diarrheal and emetic food borne illness. However, in Africa, the prevalence of food borne infections attributable to this bacterium is difficult to establish accurately. Given the involvement of farinaceous foods in the majority of emetic outbreak and the dietary habits in Ivory Coast, implying an increase in the consumption of traditionally produced flours, the prevalence of *Bacillus cereus* and emetic strains associated to local flours produced in Abidjan were assessed. In this purpose, 100 samples of millet, maize and cassava flours were collected from five different Abidjan municipalities. Microbiological analysis were carried out in accordance with the standard EN ISO 7932 : 2004 and the European regulation EC 2073/2005, to enumerate the *Bacillus cereus*.. *Bacillus cereus* strains isolated were screened by polymerase chain reaction, for emetic strains detection. Overall, 31 (31 %) of maize, millet and cassava flours were positive for *Bacillus cereus*, at levels below $10^3$ CFU/g. Comparing the five municipalities, the concentration of *Bacillus cereus* was not significantly different (P<0,05). Also, no significant difference was observed, regardless of the types of flour analyzed. Furthermore, no potentially emetic toxin–producing strains were detected in this work.

**Keyword :** *Bacillus cereus*, flour, pathogen, emetic strain.

## *RESUME*

### *PREVALENCE DE LA DETECTION DE BACILLUS CEREUS ET DE SOUCHES EMETIQUES DANS LES FARINES LOCALES DE COTE D'IVOIRE*

*Bacillus cereus est un pathogène alimentaire responsable de toxi-infection diarrhéique et émétique. Le syndrome émétique est généralement associé aux aliments farineux. Compte tenu de l'implication des aliments farineux dans les toxi-infections émétiques et de la grande consommation de farines localement produites en Côte d'Ivoire, la prévalence de* B. cereus *a été évaluée dans cette denrée. 100 échantillons de farines de mil, de maïs et de manioc ont été collectés dans cinq communes d'Abidjan. Des analyses microbiologiques ont été effectuées conformément à la norme EN ISO 7932: 2004 et à la réglementation européenne CE 2073/2005, afin de dénombrer les* Bacillus Cereus *et identifier les souches émétiques. Cet pathogène était présent dans 31 (31 %) des farines de maïs, de mil et de manioc, à des concentrations inférieures à* $10^3$ *UFC/g. Cette concentration n'est pas significativement différente (p <0,05), d'une commune à l'autre et aucune différence significative n'a été observée, quelque soit le type de farine analysé. Par ailleurs, aucune souche émétique n'a été détectée dans ces farines.*

***Mots clés :*** *Bacillus cereus, farine, pathogène, souche émétique.*

world population. These foods have been utilized from ancient times for the production of local traditional foods; now, they are introduced on international markets as innovative products and healthy foods (Wrigley *et al*., 2017; Guerrieri and Cavaletto, 2018). Millet and maize were found to be part of the mainly cereals produced worldwide (Mazoyer and Roudart, 1997; N'guessan *et al*., 2014). They play a critical role in food security on account of their agronomic characteristics, in ressource-poor developing countries.

In Africa, most children are feed with traditional porridges prepared from cereal flours, during and beyong weaning (Kimanya *et al*., 2003). Thus, studies are performed to improve the energy density and fluidity of these local porridges in order to cover the nutritional needs of the child. (Kouassi *et al*., 2015 ; Fogny *et al.,* 2017; Koné *et al*., 2019). Cereals flours are therfore found to have undeniable benefits in diet in the African population.

However, these foodstuffs are produced in traditional production units under poor sanitary conditions, susceptible to promote micro-organisms development (Motarjemi, 2002; Humblot *et al*., 2012) and foodborne disease. Even if microorganisms growth is not sustained under flour low water activity, foodborne bacteria included including *Bacillus Cereus* can easily contaminate flour and survive for long time periods. Moreover, food poisonings become increasingly attributed to *Bacillus cereus* (Mead *et al*., 1999 ; Scallan*et al*., 2011 ; Da Riol *et al*., 2018). This pathogen is mainly known to evoke two types of foodborne poisonings. The diarrheal syndrome is caused by enterotoxins produced during growth in the intestine and, the emetic type is caused by the dodeca depsipeptide cereulide pre-formed in food. Usually, both diseases are selflimiting but occasionally more severe forms, including fatal ones, are reported (Dierick*et al*., 2005 ; Naranjo *et al*., 2011 ; Tewari *et al*., 2015 ; Messelhäußer and Ehling-Schulz, 2018). Infants and babies are more susceptible to infection by such pathogens because of their less well-developed immune system and lack of competing intestinal flora (Townsend *et al*., 2008 ; Sadek *et al*., 2018). Moreover, As reported by FAO/WHO Expert Consultations, *Bacillus cereus* was among the primary microbes associated with baby food contamination (Wang *et al*., 2009).

In light of this analysis, studies on the presence of *Bacillus cereus* in flour is very valuable from the viewpoint of risk assessment. The current study aims to evaluate the prevalence of *Bacillus cereus* in local traditional flours, especially in millet, maize and cassava flours, collected in several cities in Abidjan. This quantification was performed in order to measure the dangers associated with these flours consumption.

## MATERIALS AND METHOD

100 flour samples included millet, maize and cassava, were collected from five markets in five municipalities from July to January 2019. The total number of the collected sample in different municipalities is described in table 1. Samples were conditioned in the sterile containerand held at 4°C in a cooling box, then transported to the laboratory for analysis. The number of samples decreased from 8 to 6 because of the cost of analyzes.

MOISTURE DETECTION

**Table 1 :** Flours sampling protocol.

*Protocole d'échantillonnage.*

| Municipalités | Sample numbers | | | Total numbers |
|---|---|---|---|---|
| | Maize | Millet | Cassava | |
| Yopougon | 8 | 6 | 6 | 20 |
| Adjamé | 8 | 6 | 6 | 20 |
| Abobo | 8 | 6 | 6 | 20 |
| Cocody | 8 | 6 | 6 | 20 |
| Treichville | 8 | 6 | 6 | 20 |

Moisture is an important parameter in flour that significantly affects the shelf life and growth of microbial contaminants (ICMSF, 1998). It is a very important factor in the keeping quality of flours. Moisture content measurement was established according to the official methods of AOAC International 18 th Ed. 2005. Drying in the oven of the sample under specific conditions. Weight loss is calculated as the water content of the sample.

## ENUMERATION OF BACILLUS CEREUS FROM FLOURS SAMPLES

Microbiological analyses were carried out in accordance with the EC 2073/2005 regulation. Overall, 100 millet, maize and cassava flours samples were analysed in accordance with the European regulation EC 2073/2005. The ISO 7932 and ISO 6887-1:1999 methods were used for the *Bacillus Cereus* enumeration. Flour samples (10 g) were mixed with 90 mL of buffered peptone water in stomacher bags and further homogen zed in a stomacker equipment for 1 min at 300 units.

After successive 10-fold dilutions in buffered peptone water, 100 ìl of suspensions are plated on Mannitol egg Yolk Polymyxin (MYP) agar media, following by 24h of incubation at 30°C. Then, the typical pink colonies surrounded by a zone of lecithin hydrolysis were enumerated. Each dilution was analysed three replicates.

The prevalence of *Bacillus cereus* in flours was assessed using the criteria in the Trade and Retail Federation (FCD) recommendation, what we have extended to traditional produced flours.

*Bacillus cereus* is typically mannitol-negative, produce pink-red colonies in MYP agar with a zone of precipitate around the colonies, indicating lecithinase-positive activity *Bacillus Cereus* was isolated from flours at varying levels.

**Table 2** : Microbiological criteria as recommended by the Trade and Retail Federation’s (FCD) microbiological criteria for grocery (wheat flour) : Criteria MP/MDD LS Reception / Distribution.

*Critères microbiologiques recommandés par la fédération du commerce et de la distribution (FCD) critères microbiologiques pour l’épicerie (farine de blé) : Critères MP / MDD LS Réception / Distribution.*

| Sectors | Microorganisms | n | c | Satisfatory ($m^a$) | Acceptable ($M^b$) | Unsatisfaory | Unit | European commission 2005 |
|---|---|---|---|---|---|---|---|---|
| Distribution | *Bacillus Cereus* | 5 | 2 | ≤100 | 1000 | >1000 | ufc/g | Guide values |

[a]In a three-class sampling plan, the « m » limit is used to distinguish acceptable quality units (under good manufacturing practices) from those of poor quality.

[b]The numerical value of « M » represents unacceptable concentrations of microorganisms. Exceeding the « m » level requires corrective action, i.e. revision of the HACCP plan. Exceeding the « M » value requires a recall of the product from the market.

The number of sampling units is represented by n

The maximum allowable number of sample(s) that yielded unsatisfactory test results is represented by « c »

## MOBILITY, LECITHINASE AND HAEMOLYSIS ACTIVITIES

The *Bacillus* strains were grown overnight on LB agar. Each strain was then diluted in 40 ml of saline water (0.8 5%) before spotting (3 µl) on Columbia-based blood agar (containing 5 % sheep blood) and Mannitol-egg-Yolk-Polymixin (MYP) plates. The plates were incubated 30 °C. Haemolysis and lecithinase activities were read after 24 h.

Bacterial motility was tested according to the ISO 7932:1997. For this purpose, a loopful of bacteria was inoculated centrally in glucose agar tubes and incubated at 30 °C for 24 h. A yellow colour from the central inoculation indicated a positive reaction.

### EMETIC STRAINS DETECTION

*Bacillus cereus* strains isolated from millet, maize and cassava flours were screened by PCR « polymerase chain reaction », for the presence of emetic strains. For this purpose, two distinct primer pairs (Em1F/R and CesF1/R2) targeting conserved sequence motifs of the cesB NRPS (*non-ribosomal peptides synthetase*) gene were used (Ehling-Schulz *et al.*, 2004 ; 2005) in this study.

### STATISTICAL ANALYSIS

Experimental data were compiled in Microsoft Excel 2010 software for descriptive and statistical analyses. Data were expressed as mean ± standard deviation (SD), analyzed with XL stat 2014, and then subjected to the Student test (5 % ).

## RESULTS

### MOISTURE CONTENT OF FLOURS

Moisture contents of local traditional flours are depicted in table 3. The means of maize, millet and cassava flours moisture content varied with different sampling points. i.e. from 14±0,48 to 18,52±0,22 (maize); 14,99±0,15 to 17, 21±0,33 (millet) and 11,02±0,46 to 12,33±0,8 (cassava).The moisture content varied from one municipality to another for each type of flour and from one type of flour to another within the same municipality.

**Table 3 :** Moisture content of flours from different market.

*Teneur en humidité des farines provenant des différents marchés.*

| Municipalities | Moisture content (%) | | |
|---|---|---|---|
| | Maize | millet | Cassava flour |
| Yopougon | 17±0,32 | 15,2±0,45 | 12,33±0,8 |
| Adjamé | 14±0,48 | 17, 21±0,33 | 11,41±0,44 |
| Abobo | 18,52±0,22 | 14,99±0,15 | 10,22±0,34 |
| Cocody | 16,98±0,13 | 16,42±0,21 | 12,62±0,12 |
| Treichville | 14,82±0,62 | 15, 62±0,52 | 11,02±0,46 |
| Average | 16.26±1.72 | 15.54±0.69 | 11.52±0.92 |

### *BACILLUS CEREUS* ENUMERATION FROM FLOURS

This bacterium is typically mannitol-negative, produce pink-red colonies in MYP agar with a zone of precipitate around the colonies, indicating lecithinase-positive activity. *Bacillus cereus* was isolated from flours at varying levels. Out of a total of 100 flour samples collected, 13 % (Yopougon), 10 % (Adjamé), 10% (Abobo), 7% (Cocody) and 11% (Treichville) were without detectable levels of *Bacillus cereus* according to municipalities (table 4). However, a total of 30 %, 23.33 % and 40 % of maize, millet and cassava flours were respectively contaminated by *Bacillus cereus*, at levels from 1 x $10^1$ CFU/g to 1 x $10^2$ CFU/g.

**Table 4 :** Level of *Bacillus Cereus* in different flours collected.

*Nombre de* Bacillus Cereus *dans les différents types de farines.*

| Sales woman | Municipalities | | | | |
|---|---|---|---|---|---|
| | Yopougon | Adjamé | Abobo | Cocody | Treichville |
| | *Bacillus Cereus* in maize flour (UFC/g) | | | | |
| 1 | 2,20E+02 | 0,00E+00 | 4,00E+01 | 0,00E+00 | 4,00E+02 |
| 2 | 3,00E+01 | 0,00E+00 | 6,00E+01 | 0,00E+00 | 8,00E+01 |
| 3 | 0,00E+00 | 0,00E+00 | 0,00E+00 | 2,00E+02 | 0,00E+00 |
| 4 | 2,70E+02 | 0,00E+00 | 4,00E+01 | 6,00E+01 | 3,00E+01 |
| 5 | 0,00E+00 | 5,00E+01 | 1,60E+02 | 7,00E+01 | 0,00E+00 |
| 6 | 0,00E+00 | 1,60E+02 | 2,80E+02 | 1,10E+02 | 0,00E+00 |
| 7 | 0,00E+00 | 0,00E+00 | 0,00E+00 | 2,60E+02 | 3,00E+01 |
| 8 | 2,10E+02 | 0,00E+00 | 1,40E+02 | 1,40E+02 | 0,00E+00 |
| Average | 9.13E+01 | 2.63E+01 | 9.00E+01 | 1.05E+02 | 6.75E+01 |
| | *Bacillus Cereus* in millet flour (UFC/g) | | | | |
| 1 | 0,00E+00 | 0,00E+00 | 0,00E+00 | 1,90E+02 | 0,00E+00 |
| 2 | 0,00E+00 | 4,00E+01 | 0,00E+00 | 2,60E+02 | 0,00E+00 |
| 3 | 0,00E+00 | 0,00E+00 | 0,00E+00 | 1,80E+02 | 3,20E+02 |
| 4 | 0,00E+00 | 3,00E+01 | 0,00E+00 | 0,00E+00 | 0,00E+00 |
| 5 | 0,00E+00 | 0,00E+00 | 0,00E+00 | 4,00E+01 | 0,00E+00 |
| 6 | 0,00E+00 | 3,20E+02 | 3,60E+02 | 0,00E+00 | 2,60E+02 |
| Average | 0.00E+00 | 6.50E+01 | 6.00E+01 | 1.12E+02 | 9.67E+01 |
| | *Bacillus Cereus* in cassava flour (UFC/g) | | | | |
| 1 | 5,00E+01 | 3,20E+02 | 0,00E+00 | 1,40E+02 | 3,90E+02 |
| 2 | 0,00E+00 | 3,90E+02 | 4,00E+01 | 1,00E+02 | 2,80E+02 |
| 3 | 0,00E+00 | 0,00E+00 | 0,00E+00 | 8,00E+01 | 2,30E+02 |
| 4 | 0,00E+00 | 1,70E+02 | 0,00E+00 | 0,00E+00 | 0,00E+00 |
| 5 | 2,10E+02 | 2,30E+02 | 4,10E+02 | 0,00E+00 | 0,00E+00 |
| 6 | 3,70E+02 | 6,00E+01 | 2,50E+02 | 0,00E+00 | 0,00E+00 |
| Average | 1.05E+02 | 1.95E+02 | 1.17E+02 | 5.33E+01 | 1.50E+02 |

## EMETIC STRAIN DETECTION

60 presumptive *Bacillus Cereus* cereulide-producing strains from locale and traditionally produced flours were, isolated regard less of the type of flours (maize, millet and cassava flours) samples and investigated by PCR for the presence of ces-relatedgenes. Among the 60 strainstested, none emetic strain was identified by PCR.

# DISCUSSION

## MOISTURE CONTENT OF FLOURS

Moisture content in our study ranged from 10,22±0,34 to 18,52±0,22. But the stipulated limit is 15 % (WFP, 2012). Our results are thus above the recommended maximum values and above the moisture content determined in other studies (N'tuli *et al*., 2013) in some municipalities. According to Ijah *et al*., (2014), these different moisture contents could be linked to the processing methods the samples were exposed to. Indeed, The process of drying foods not only affects the water content of the products, but also other physical and chemical characteristics such as colour, flavour and texture. Moisture content of multi-cereals exposed to sun drying was found to be greater when compared to fluidized bed drying and forced convection tray drying (Rahman, 1999 ; Kumari *et al*., 2017).In the currentstudy, high moisture content of the flours analyzed could be explained by sundrying and milling process with local small-scale producers. Thishigh moisture content might limited flours shelf life.

## BACILLUS CEREUS IN FLOURS

The prevalence of *Bacillus cereus* in different flours could be explained by their moisture contents, the abilities of this bacterium to survive under high temperature and dried conditions as well as to the packaging process of this type of food (Claus and Berkeley, 1986; Väisänen *et al*., 1991 ; Amor *et al*., 2018). Furthermore, production and marketing of local flours in Abidjan is done traditionally in general and under unhygienic conditions. This could favor the bacteria development, included *Bacillus cereus*, in these flours. The prevalence of *Bacillus cereus* in different cereal flours were described elsewhere. Thus, in the study conductedby Amor *et al*., (2018), an amount of *Bacillus cereus* group bacteria, ranged from 30 to more than 2.4 $10^3$ MPN/g was isolated from cereal flour.

Berghofer *et al*., (2003), have also noted a significant degree of incoming wheat and wheat flour samples contaminated by *Bacillus cereus*. According to these authors, the field contamination and the presence of *Bacillus cereus* spore in milling equipment, conta-minating other wise 'clean' product. In (2017), Zhang *et al.* have reported *Bacillus cereus* content in flour infant formula and rice flour samples. They found that 40 out of the 587 samples analyzed, were positive for *Bacillus Cereus*, at level seven attained $10^3$–$10^4$CFU/g in four infant formula samples and one rice flour sample. These results are higher to those obtained in this study, where as the results of Eglezos (2010) are lower to ours. Indeed, this authorinve stigated the microbiological quality of grain and wheat flour from two plants in Australia and reported a prevalence of *Bacillus cereus* of < 0.3% in wheat flour, with an average of 0.8 log CFU/g in the positive samples.

In addition to moisture, susceptible to promote the *Bacillus cereus* development, the prevalence of this bacterium in our study could be explained by the fact that most women producers are low-income and not educated women, who carry out this activity to support their families. They therefore pay less attention to hygiene during production. Furthermore, the promiscuity of the drying sites of the flours, linked to the precarious housing of the producers does not allow a better maintenance of the drying sites (Yobouet *et al*., 2016).

Comparing the five municipalities, the levels of *Bacillus cereus* were not significantly different (P < 0,05). Besides, no significant difference was observed, regard less of the types of flouranaly-zed.The risk of contamination by *Bacillus cereus* could be the same for the populations of the five municipalities investigated.

## EMETIC STRAIN DETECTION

Our results on the absence of emetic strains among *Bacillus cereus* isolates confirm previous studies that have shown the scarcity of emetic strains in the environment. (Altayar and Sutherland, 2006). Further more, the true incidence of emetic strains might still be under estimated since emetic strains are only weakly or non-hemolytic (Ehling-Schulz *et al.,* 2005 ; Pirhonen *et al*., 2005) and thus might be misdiagnosed as being not *Bacillus cereus*. Moreover, it has been shown that non-hemolytic strains were discarded and emetic strains were found to be absent or extremely rare in studies on *Bacillus cereus* prevalence in food (Rosenquist *et al.*, 2005 ; Ankolekar *et al.*, 2009 ; Samapundo *et al.*, 2011 ; Shling-Schulz *et al.*, 2015). However, despite this scarcity, several fatal cases of foodborne infection have been reported, owever, despite this scarcity, several fatal cases of foodborne infection have been reported, showing the hazardousness s of this bacterial strain (Messelhäußer and Ehling-Schulz, 2018).

## CONCLUSION

This study was conducted at a time when few investigations related to the prevalence of *Bacillus Cereus* in Ivory Coast has been done. Although no emetic strain was detected in maize, millet and cassava flours traditionally produced in Abidjan, *Bacillus Cereus* count was isolated from them. Even if in low levels, this bacterium is able to develop and cause food borne illness, as indicated by reported outbreaks. Given the undeniable benefits in diet in the African population of the above cereal flours, it would be recommended to sensitize the flour producers to work under hygienic conditions as well as to respect the storage temperature which is the most important factor in keeping *Bacillus cereus* numbers to a minimum. Additional studies to expand the scope of our investigation to other municipalities are needed.

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