Daily Intake of Aflatoxins from Cocoa (Theobroma cacao) Product in Cote D'Ivoire

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Abstract: The objective of this study is to estimate the risk of exposure to aflatoxins cocoa bean (Theobroma cacao) produced in Côte d'Ivoire. Thus, 113 samples (66 samples collected during the 2011-2012 season and 47 during that of 2012-2013) were analyzed. Aflatoxin was extracted from the samples by the modified method of Le Tutour et al., (1983) and assayed by high performance liquid chromatography (HPLC) according to the standards of the European Communities (EC 401/2006). Aflatoxin intakes were estimated from daily consumption of cocoa beans from the adult Ivorian and levels of aflatoxins found in the cocoa mass. The mean concentrations of aflatoxin B1 (AFB1) and total aflatoxin (AFT) found in the cocoa mass were respectively 1.31±0.48 µg/kg and 2.36±0.61 µg/kg. The AFB1 was found in 58% of samples with higher contents to normative values of the European Commission in 20% and 16% respectively for AFB1 (2 µg/kg) and AFT (4 µg/kg). The estimated intakes for AFB1, are 25.2 pg/kg body weight/day and 266.5 pg/kg body weight/day, respectively for small and large Ivorian consumers. These calculated contributions represent 16.8% and 177.7% of the Toxicity Reference Value (VTR) stable by European and international committees. According to the VTR, the levels of aflatoxins detected in the mass cocoa are not likely to cause toxicity to the small Ivorian consumer. However, health risks remain for regular Ivorian consumer because of the potential synergy of toxins and exposure over long periods at these doses determined.

Keywords: aflatoxins, cocoa mass, health risk, daily intake

1. Introduction

Aflatoxin B1, B2, G1 and G2 are mycotoxins produced by molds of the genus Aspergillus [1]. The fear caused by these aflatoxins is from the danger they pose to humans and animals. Indeed, the primary target organ for aflatoxins is the liver [2]. They can also disrupt the immune system, cause growth retardation and possibly death in humans and pets [3]-[6]. Aflatoxin B1 is also teratogenic, immunosuppressive and could affect reproductive functions [7]. It was also proved that aflatoxins significantly slow the recovery of child victims of protein-calorie malnutrition [8]. Furthermore, exposure to high amounts of aflatoxin, at weaning, can affect the growth of children as some authors have pointed to Benin and Togo [9], [10].

The presence of aflatoxins alters the health quality of contaminated food (nuts, corn, peanuts, oil seeds, spices, dried fruits, cocoa products) [11]. In 2004, aflatoxins have been responsible for the deaths of 80 people in Kenya following the consumption of corn flour heavily contaminated aflatoxin [12]. Aflatoxin B and G are mainly found in the cake and a minority in the crude oil [7].

The cocoa tree (Theobroma cacao L.), the Malvaceae family is a tropical plant cultivated for its beans which are extracted powder and cocoa butter [13]. Cocoa is an important source of income for small farmers, who are responsible for the bulk of world production [14]. Cocoa production is also a source of foreign currency for producer countries including Côte d'Ivoire. Indeed, the Côte d'Ivoire is the world's largest cocoa producer with about 40% of world production. This sector accounts for 15% of gross domestic product and exports of processed cocoa and cocoa beans represent nearly 45% of export earnings of the country [15], [16]. It employs about 600 000 farmers and is the source of income for more than a third of the Ivorian population. Also this sector occupies 40% of the country's banking sector activities [17]. The importance of this crop for both sectors directly or indirectly bound to it, in fact a lever for economic development and poverty reduction. However, only 35% of Ivorian cocoa is now processed locally [15].

Ivorian cocoa is exported to Europe and North America as beans, cocoa butter and cocoa powder [18], [19]. The exported products such analysis indicates that the exported proportions are respectively 69.1%, 29.9% and 1.0% for beans, derivatives and waste [19]. Tagro et al. (2007) identified in the Ivorian cocoa, Aspergillus species such as Aspergillus flavus [20]. This species is suspected to produce aflatoxins [1]. However in Côte d'Ivoire the emphasis is on the presence of ochratoxin A in cocoa and derived products to the detriment of aflatoxins [21]-[24]. Furthermore, the presence of aflatoxins in cocoa and derivatives has been reported in Japan [25], in Turkey [26] and Brazil [27], [28].

This study was initiated to determine the level of contamination and estimate the daily intake of aflatoxins from crushed cocoa beans (cocoa mass), produced in Côte d'Ivoire, after roasting and shelling.
2. Material and Methods

2.1 Sampling

The plant material used consists of shredded cocoa beans, obtained after roasting and husking (cocoa mass), collected during the 2011-2012 and 2012-2013 crops from processors of cocoa beans. Thus 66 and 47 samples were taken respectively, according to regulation 401/2006 / EC [29], during crops 2011-2012 and 2012-2013.

2.2 Determination of aflatoxins

2.2.1. Extraction method of aflatoxins

In a 250 mL Erlenmeyer flask containing 20 g of cocoa mass, 100 ml of methanol-water (v/v, 8:2) were added. The mixture was homogenized by shaking for 2 minutes and then stored at room temperature in the dark for 12 hours. The homogenate was filtered through filter paper and aflatoxins were extracted from the filtrate with 3 volumes of 10 ml of chloroform [30]. The extracts were collected and evaporated to dryness using a rotary evaporator at 40°C. A dry extract were added 0.4 mL of hydrochloric acid and 4.6 mL of bidistilled water. The mixture was filtered through filter paper and aflatoxins were extracted by a liquid chromatograph HPLC brand Shimadzu coupled to a fluorescence detector was used and the operating conditions are described in Table 1.

Table 1: Operating conditions of HPLC analysis

<table>
<thead>
<tr>
<th>Pre column</th>
<th>Shim-pack GVP-ODS 10 x 4.6 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Shim-pack GVP-ODS 250 mm x 4.6 mm</td>
</tr>
<tr>
<td>Detector</td>
<td>Fluorescence; λ excitation: 365 nm, λ emission: 435 nm</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Water / acetonitrile / methanol (6/2/2, v/v/v)</td>
</tr>
<tr>
<td>Injection volume</td>
<td>20 µL</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 mL / min gradient</td>
</tr>
<tr>
<td>Temperature of the Column</td>
<td>40°C</td>
</tr>
<tr>
<td>Rinsing solvent</td>
<td>Methanol</td>
</tr>
<tr>
<td>Duration of analysis</td>
<td>13 minutes</td>
</tr>
<tr>
<td>Limit of Detection (LD : ng/kg)</td>
<td>AFB1: 5±2, AFB2: 2±1, AFG1: 3±1, AFG2: 4±2</td>
</tr>
<tr>
<td>Limit of quantitation (LQ : ng/kg)</td>
<td>AFB1: 30±10, AFB2: 40±20, AFG1: 40±20, AFG2: 30±10</td>
</tr>
<tr>
<td>Recovery rate (%)</td>
<td>79±0.9, 82±0.8, 85±0.6, 80±0.8</td>
</tr>
</tbody>
</table>

2.2.2. Equipment

A liquid chromatograph HPLC brand Shimadzu coupled to a fluorescence detector was used and the operating conditions are described in Table 1.

2.3 Estimated daily intake of aflatoxins from mass cocoa

Deterministic approach was adopted to assess exposure to aflatoxins. This approach consists in multiplying a fixed value of food consumption by a fixed value of the contamination and to divide the consumption by the actual body weight of the individual [31], [32]. The aflatoxin intake was calculated using the formula 1.

\[
AAF = \frac{(\text{Conc AF} \times \text{Qtep})}{\text{Mc}}
\]  

AAF: daily intake of aflatoxin Pg/kg Pc/day for a 70 kg individual  
Conc AF: concentration of aflatoxins in pg/kg of product Qtep: amount of product consumed in kg/day  
Mc: body weight in kg

According to the International Cocoa Organization, the daily consumption of cocoa for the Ivorian adult is 1.345 g/capita/day [33]. On the one hand, estimates intakes were obtained for average and maximum contamination levels and, on the other hand to the maximum tolerated concentrations of 2 µg/kg and 4 µg/kg for AFB1 and AFT respectively [34]. The estimated intakes were compared to the VTR (DJTP equal to 150 pg /kg/day) established by the European and International Scientific Committees [35]-[38]. The results were expressed as the percentage contribution of the VTR for B1 and total aflatoxins.

2.4 Statistical analysis

The averages were calculated with confidence intervals to assess the level of contamination in aflatoxins. The percentages have allowed to assess the occurrence of aflatoxins in mass cocoa samples and the proportions of the estimated intakes of aflatoxins compared to the VTR. The homogeneity of the averages (aflatoxins) was evaluated by analysis of variance with a factor (sampling period), through the F test, from software XLSTAT 7.5 and Excel at risk 5%.

3. Results

3.1 Measuring the concentrations of aflatoxins

The average concentration of aflatoxin B1 (1.31 µg/kg) and maximum concentration (13.87 µg/kg) were observed during the campaign 2011/2012. For AFB2, the average concentration is 0.31 µg/kg. Regarding the AFG 1 and AFG2, average concentrations are 0.31 µg/kg and 0.43 µg/kg respectively. The results show that AFB1, AFB2, AFG1 and AFG2 were quantified respectively in 58%, 52%, 36% and 58% of the 113 samples analyzed (Table 2).
Table 2: Concentrations and variation field of aflatoxins and proportion of positive samples

<table>
<thead>
<tr>
<th>Crops</th>
<th>AFB1</th>
<th>AFB2</th>
<th>AFG1</th>
<th>AFG2</th>
<th>AFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011-2012</td>
<td>1.73±0.69</td>
<td>0.32±0.12</td>
<td>0.29±0.10</td>
<td>0.64±0.24</td>
<td>2.97±0.86</td>
</tr>
<tr>
<td>&lt;0.005-13.87</td>
<td>a</td>
<td>[ &lt;0.002-2.82]</td>
<td>[ &lt;0.003-3.18]</td>
<td>[ &lt;0.004-7.08]</td>
<td>[nd-17.48]</td>
</tr>
<tr>
<td>2012-2013</td>
<td>1.01±0.32</td>
<td>0.31±0.08</td>
<td>0.33±0.12</td>
<td>0.28±0.07</td>
<td>1.92±0.31</td>
</tr>
<tr>
<td>&lt;0.005-5.33</td>
<td>a</td>
<td>[ &lt;0.002-1.86]</td>
<td>[ &lt;0.003-3.83]</td>
<td>[ &lt;0.004-7.08]</td>
<td>[nd-7.34]</td>
</tr>
<tr>
<td>All samples</td>
<td>1.3±0.48</td>
<td>0.31±0.10</td>
<td>0.31±0.12</td>
<td>0.43±0.17</td>
<td>2.36±0.61</td>
</tr>
<tr>
<td>&lt;0.005-13.87</td>
<td>a</td>
<td>[ &lt;0.002-2.82]</td>
<td>[ &lt;0.003-3.83]</td>
<td>[ &lt;0.004-7.08]</td>
<td>[nd-17.48]</td>
</tr>
</tbody>
</table>

Table 4: Daily intake of aflatoxins from cocoa mass in adult Ivorian (Intake in pg/kg body weight/day)

<table>
<thead>
<tr>
<th>Crops</th>
<th>AFB1</th>
<th>AFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated intake from LMA of AFB1</td>
<td>38.43</td>
<td></td>
</tr>
<tr>
<td>Estimated intake from LMA of AFT</td>
<td>76.86</td>
<td></td>
</tr>
<tr>
<td>Reference value SCF and JECFA</td>
<td>150</td>
<td></td>
</tr>
</tbody>
</table>

2.4. Comparison of aflatoxin intake from mass cocoa with reference values

For an average consumer, AFB1 and AFT intakes from mass cocoa represent 65.5% and 59.0%, respectively of intakes calculated from the LMAB1 and LMAT. Intakes of AFB1 and AFT in cocoa mass represent respectively 16.8% and 30.2% of VTR. Regarding the large consumer, intakes of AFB1 and AFT from cocoa mass represent 693.5% and 437.0%, respectively of the intakes calculated from LMAB1 and LMAT. Intakes of AFB1 and AFT from cocoa mass represent respectively 177.7% and 223.9% of VTR (Table 5).

Table 5: Comparison of aflatoxins intakes from mass cocoa with reference values (%)

<table>
<thead>
<tr>
<th>Crops</th>
<th>AFB1</th>
<th>AFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMAB1</td>
<td>65.5</td>
<td>346.2</td>
</tr>
<tr>
<td>LMAT</td>
<td>32.7</td>
<td>346.7</td>
</tr>
<tr>
<td>VTR</td>
<td>16.8</td>
<td>177.7</td>
</tr>
</tbody>
</table>

2.2. Proportion of samples non-compliant with European standards

Table 3 shows that 20% of samples have AFB1 concentrations above 2 µg/kg. For AFT, 16% of samples have concentrations greater than 4 µg/kg. The maximum permissible limits (LMA) are 2 µg/kg for AFB1 and 4 µg/kg for AFT.

2.3. Daily intake of aflatoxin from mass cocoa in an adult Ivorian

Aflatoxin intakes estimated for an Ivorian average consumer are 25.17 pg/kg body weight/day and 45.35 pg/kg body weight/day, respectively for AFB1 and AFT. While those determined for a large Ivorian consumer are 266.5 pg/kg body weight/day and 335.8 pg/kg body weight/day, respectively for AFB1 and AFT. Intakes determined from Authorized Maximum Limits (LMA) are 38.43 pg/kg body weight/day and 76.86 pg/kg body weight/day, respectively for AFB1 and AFT. For an average consumer, AFB1 and AFT intakes are lower than those determined from the LMA and VTR established by the Scientific Committees European and international (JECFA and SCF). As against for a large consumer, the determined intakes are much higher than those estimated from the LMA and VTR (Table 4).

2.3. Daily intake of aflatoxin from mass cocoa in an adult Ivorian

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2011-2012 crop. This decrease could be explained by the implementation of the reform of the coffee-cocoa sector. According to the Coffee and Cocoa Board, the benefits of advocacy and technical assistance to farmers have contributed to improving the quality of production. Specifically, this resulted in the decrease of the moisture content of cocoa beans from 12% to 8% and the rate of foreign material in crops by 3% to 1.1% between 2012 and 2013 [15], [40]. According to work by Kouadio et al. (2015) higher the moisture content of cocoa beans is important higher the percentage of contamination (fungus and mycotoxins) increased [41].

For an Ivorian average consumer, intake of AFB1 calculated represents 65.5% and 16.8% of the contribution calculated from LMA set by the European Commission [34] and VTR [35]-[38] respectively. Concerning AFT, this intake represents 59.0% and 30.2% of the calculated intake from the LMA [34] and the VTR [35]-[38] respectively. High intakes of aflatoxins were calculated from other power supplies. In 1998, the estimated average intake for French population in AFB1 and AFT are respectively 130 pg/kg body weight/day and 120 pg/kg body weight/day [42]. These intakes of AFB1 and AFT represent 86.7% and 80% of the VTR respectively. Under this standard on VTR, aflatoxin levels detected in the Ivorian cocoa mass are safe for an average consumer [35]-[38]. However, health risks related to the synergy of toxins remain [39], [43]-[46]. In fact, fixing these health standards guarantee an acceptable level of food security. But it is essential to mention the difficulty of estimating the risks of repeated ingestion of aflatoxins in the medium and long-term. The exposure over long periods at very low doses and toxic interactions occurring between mycotoxins and other pathogens may have health consequences of this consumer. As regards a large Ivorian consumer, intake of AFB1 calculated represents 693.5% and 177.7% of intake calculated from LMA [34] and VTR [35]-[38] respectively. As to AFT, this intake represents 437.0% and 223.9% of calculated intake from LMA [34] and VTR [35]-[38] respectively. These results indicate that cocoa can be considered a risk food to consumers exceptional. Indeed, SCF (1994) and JECCA (1999) concluded that exposure levels less than 1 ng of aflatoxins/kg body weight/day always contribute to risk of liver cancer [35], [37].

CONCLUSION

This study has clearly showed the presence of AFB1 and AFT in mass cocoa produced in Côte d’Ivoire. This mycotoxin was quantified in over half of samples collected. The results show that the daily intakes estimated of AFB1 and AFT exceed the VTR of aflatoxins in Ivorian regular consumers of cocoa. Thus this study indicates the need for periodic monitoring of the occurrence of aflatoxins in cocoa mass and other dry products (cereals, cocoa beans, green coffee, peanuts and cashews). Hence, because of the carcinogenic, genotoxic without threshold of this mycotoxin, the only realistic approach is to reduce exposure to as low a level as possible following the ALARA (As Low As Reasonably Achievable).

References


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