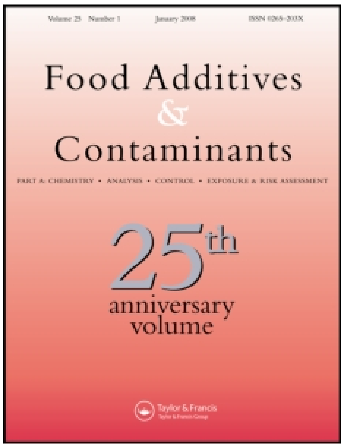

Food Additives & Contaminants: Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713599661

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To cite this Article Manda, Pierre, Dano, Djédjé Sébastien, Kouadio, James Halbin, Diakité, Aïssata, Sangaré-Tigori, Béatrice, Ezoulin, Miezan Jean Marc, Soumahoro, Awa, Dembele, Ardjouma and Fourny, Gérard(2009) 'Impact of industrial treatments on ochratoxin A content in artificially contaminated cocoa beans', Food Additives & Contaminants: Part A, 26: 7, 1081 — 1088

To link to this Article: DOI: 10.1080/02652030902894397

URL: http://dx.doi.org/10.1080/02652030902894397

Food Additives and Contaminants
Vol. 26, No. 7, July 2009, 1081–1088

Taylor & Francis
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Impact of industrial treatments on ochratoxin A content in artificially contaminated cocoa beans

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(*Received 22 December 2008; final version received 4 March 2009*)

Ochratoxin A (OTA) is a mycotoxin mainly produced by mould species of the genera *Aspergillus* and *Penicillium*, which grow on a variety of agricultural products. OTA-contaminated foodstuffs pose a major health hazard to consumers, including human and animal. In Côte d'Ivoire, numerous studies are being carried out to find the best way of preventing OTA contamination of cocoa raw material. The objectives of this investigation were to assess the impact of industrial treatment on OTA content in cocoa-derived products. Samples of cocoa pods were prepared under specific conditions promoting fungal proliferation on cocoa beans before processing. The beans underwent the usual industrial treatments – roasting, shelling, crushing, pressing and additive addition – and samples were taken at each stage. OTA was extracted with a methanol/3% sodium hydrogen carbonate solution and purified using an immunoaffinity column prior to HPLC analysis with fluorescence detection. OTA was detected in artificially contaminated cocoa beans at levels ranging from 3.4 to 44.7 $\mu g\,kg^{-1}$ with a mean value of $22.9 \pm 3.6\,\mu g\,kg^{-1}$. OTA was mainly concentrated in the shell (93%). Roasting, shelling and additive addition significantly decreased levels of OTA by 24–40, 76 and 52%, respectively, with an overall reduction of ~91%. These results indicate that industrial processing of cocoa has a real impact on the reduction of OTA in final cocoa products.

Keywords: ochratoxin A; cocoa; industrial processing; HPLC; Côte d'Ivoire

Introduction

The mycotoxin, ochratoxin A (OTA), is a secondary metabolite of low molecular-weight, produced by several fungi species belonging to the *Aspergillus* and *Penicillium* genera (Creppy 2002; Suarez-Quiroz et al. 2005). These toxicogenic moulds grow naturally on various agricultural products, such as cereals, oilseeds, nuts, coffee and cocoa (Iavicoli et al. 2002; Biffi et al. 2004; Sangare-Tigori et al. 2006a). Throughout the world, mycotoxins pose a major public health concern as the consumption of OTA-contaminated foodstuffs can result in a series of human and animal pathologies (Rutqvist et al. 1978; Muller et al. 1995; Creppy 2002; Pfohl-Leszkowicz and Manderville 2007). It is well established that OTA has genotoxic, immunotoxic, embryotoxic, teratogenic, carcinogenic and powerful nephrotoxic properties (Joint Expert Committee for Food Additives 2001; Creppy 2002; Weidenbach and Petzinger 2004). Moreover, observational studies have associated OTA with the aetiology of Balkan endemic nephropathy (O'Brien and Dietrich 2005; Fuchs and Peraica 2005). In Côte d'Ivoire, a study revealed that apparently healthy people had OTA in their blood in a concentration ranging from 0.01 to 5.81 $\mu g\,l^{-1}$ (Sangare-Tigori et al. 2006b).

The health risk from OTA contamination of food has led to worldwide preventive action to protect the consumer. Food quality must meet a set of directives and regulations on OTA content before being allowed into European markets (European Union 2005). Currently, the European Union (EU) has not set a maximum allowable limit for OTA in cocoa; however, it is considering new regulations, which will set the maximal amount at 2 $\mu g\,kg^{-1}$ (Working Document of the Expert Committee 2003). The enforcement of such a regulation may upset the economic balance of many developing countries, which produce and export cocoa to European markets. Côte d'Ivoire, the premier producer of cocoa (1.4 million tons per year) and ranked eighth of coffee production (0.32 million tons per year) is concerned about the potential impact of these control measures. Investigations are needed not only to develop good agriculture practices to improve the quality and safety of these top export raw materials

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ISSN 0265–203X print/ISSN 1464–5122 online

DOI: 10.1080/02652030902894397
http://www.informaworld.com

but also to assess the effectiveness of post-harvest treatment on the reduction of OTA levels in derived products. In 2005, our laboratory undertook a study regarding the incidence of roasting on OTA content in coffee. The resultant data demonstrated that coffee roasting at temperatures ranging from 190 to 210°C for 20–30 min led to a decrease of OTA levels by 73% (Dano et al. 2009).

Industrially, roasting is the first step of cocoa processing for derived products, i.e. cocoa powder, cocoa butter and chocolate; subsequent steps include shelling, crushing, pressing and additives addition. These industrial operations may play an important role in the reduction of OTA levels in cocoa derivative products.

Using a contamination model, the present work is designed to investigate the impact of each industrial treatment step, as well as their overall effect, on OTA levels in cocoa-derived semi-finished and finished products, such as cocoa powder, cocoa butter and chocolate.

Material and methods

Apparatus

A Shimadzu HPLC LC 10AD VP equipped with a Shimadzu RF–10A XL fluorimetric detector and a Shimadzu C6R 8A integrator was used to measure OTA levels. A Selecta laboratory oven was used to roast cocoa beans and a Pascal Engineering chocolate machine to process the beans into chocolate.

Reagents

Standard solution of OTA (100 ng ml^{-1}), immunoaffinity columns (IAC) and saline pastilles, used to prepare the PBS buffer solution, were purchased from R-Biopharm (Lyon, France). The reagents were obtained from different commercial sources: methanol (Prolabo), acetonitrile/HPLC, toluene for analysis (Scharlau), sodium bicarbonate (Merck) and acetic acid (SDS).

Cocoa treatment to promote mould proliferation: OTA artificial contamination

Ripe cocoa pods (heap of 500 pods) were harvested in an experimental plot of the National Centre for Agricultural Research (NCAR) located 230 km from Abidjan during the period from November 2005 to February 2006. Using a machete, deep slits were made in the outer shell of 250 pods. All pods were then left in the open air for 7 days and hulled to collect the beans combined with the whole mass of white soft pulp. Afterwards, the beans were gathered on banana leaves, covered with a black tarpaulin and left to ferment for 4 days to completely turn mouldy. Finally, the beans were sun-dried for another 7 days.

Industrial processing of cocoa

After the artificial contamination, the mouldy cocoa beans underwent industrial treatment consisting of roasting, shelling, crushing, pressing and additives addition (Figure 1). Cocoa beans were processed as semi-finished and finished products using the chocolate machine. Cocoa beans (500 g) were roasted at 140°C for 30 min in the oven, cooled and manually shelled; the nibs were separated from shells, ground and divided into two groups. One group was used to extract cocoa butter under high pressure at 50–60°C. Roasted and crushed cocoa nibs (200 g) from the second group were ground in the mortar of the chocolate machine at 60°C for 30 min. Afterwards, 180 g of the resultant paste was mixed with 180 g of sugar (icing sugar) and refined. Then, additives, including cocoa butter and milk, were added, which represent 13.4 and 10.5% of the initial mixture, respectively. Chocolate was obtained after moulding the cocoa paste. As illustrated in Figure 1, sampling was carried out during cocoa processing to determine OTA levels.

Extraction, detection and quantification of OTA

Approximately 200 g of beans and nibs were thinly ground. Cocoa pastes and chocolate pieces were ground in a porcelain mortar before taking samples. Then, 15 g of each sample were put in a Waring blender bowl and 150 ml of an aqueous solution (50 : 50, v/v) of methanol/sodium hydrogen carbonate 3% (m/v) were added and the mixture stirred for 2 min. After decanting and filtering using Whatman paper no. 4, 11 ml of filtrate was added to an equivalent volume of PBS buffer.

The immunoaffinity column was pre-conditioned with 10 ml of PBS buffer at a flow-rate of 3 ml min^{-1}. Then, 20 ml of extract was taken and loaded onto the immunoaffinity column at a flow-rate of 1–2 ml min^{-1}. OTA present in the samples was captured by the antibodies contained in the agar suspension. The immunoaffinity column was washed with 20 ml of PBS buffer to remove non-specific components.

OTA was slowly eluted by 1.5 ml of a mixture of acetic acid/methanol (2 : 98, v/v) at a rate of 1–2 drops s^{-1}. Then, the column was washed with 1.5 ml of distilled water to obtain a final volume of 2.8 ml. After stirring, analysis was performed by HPLC.

HPLC analysis was carried out in an isocratic mode using fluorimetric detection at excitation and emission wavelengths of 333 and 460 nm, respectively. The mobile phase was a mixture of acetonitrile/water/

glacial acetic acid (55 : 43 : 2, v/v) and the stationary phase was a C_{18} S5 ODS 2.5 µm (25 cm × 4.6 mm) column equipped with a pre-column. The peak of OTA in samples was identified by comparison with standards. OTA was quantified by measuring the peak area, taking into account the dilution performed during OTA extraction and purification.

Statistical analysis

Data were expressed as mean ± SEM. The occurrence of OTA in samples before and after the industrial treatments was compared using a Wilcoxon matched-pair test. Statistical significance was assumed at $p < 0.01$.

Results and discussion

Evaluation of the analytical method

The method for OTA analysis proved acceptable for cocoa matrix. OTA extraction was performed in alkaline conditions in accordance with the method described previously (Tafuri et al. 2004; Amézqueta et al. 2005). This method ensured very good recovery in our study at each spiking level. Three different samples spiked with OTA at 5, 10 or 20 µg kg^{-1} were analysed on the same day. The limit of detection (LOD) and limit of quantification (LOQ) were 0.05 and 0.2 µg kg^{-1}, respectively. It is worth noting that recovery was only carried out using unroasted cocoa beans. The average recoveries were 95.83 ± 0.70, 94.78 ± 2.05 and 95.06 ± 0.85% for OTA levels of 5, 10 and 20 µg kg^{-1}, respectively, with $n = 3$ at each level. Recoveries were very consistent and RSDs were lower than 3%, which demonstrates the precision of the analytical procedure. The method can be viewed as valid according to Directive 2002/26/CE, which indicates that between 1 and 10 µg kg^{-1} recoveries are acceptable within the range 70–110%. All data were corrected according to the overall recovery (95.2 ± 0.5%). When constructing dose–response curves for OTA analysis, solutions containing 1, 5, 10, 20 and 40 µg kg^{-1} were measured and the coefficient of linearity (r^2) was 0.9995. However, due to possible underestimation of levels of OTA, some authors recommend operating under acidic conditions (Pfohl-Leszkowicz et al. 2004, 2006; Molinié et al. 2005). The data related to recovery, relative standard deviations (RSDs) and range are displayed in Table 1.

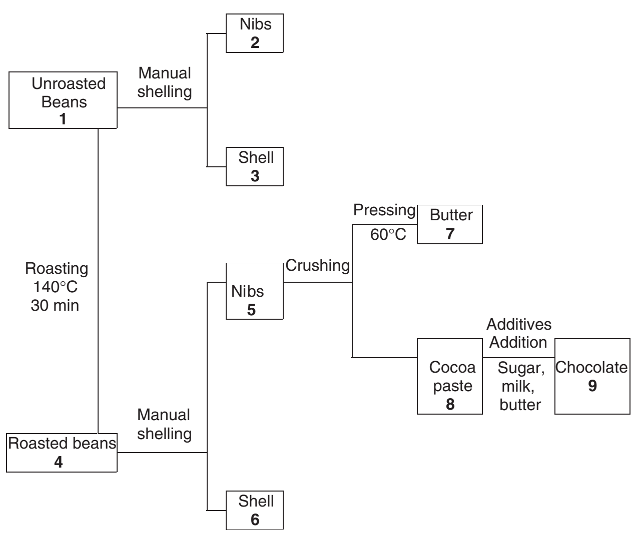

Figure 1. Summary of cocoa industrial processing steps. Numbers 1–9 represent the different stages of sampling.

Artificial contamination of cocoa beans

As OTA is a major public health concern, with serious economic consequences for developing countries, our investigation was conducted to determine the effect of industrial treatment, such as roasting, shelling and additives addition, on OTA content in cocoa products. To adequately assess the impact of these industrial operations, we used our contamination model, which consisted of preparing cocoa beans in such a way that we reached high OTA contamination levels. As described above, deep slits were made in the outer shell of cocoa pods, which were left in the open air for 7 days. This favoured fungal penetration and proliferation inside the pods and consequent OTA formation. As a result, contrary to contaminations

Table 1. Precision and recovery of the analytical procedure.

OTA added (µg kg^{-1})	OTA measured (µg kg^{-1})	Recovery (%)	Recovery average (%)	RSD (%)	Global recovery (%)
1	0.9652	96.52	95.83 ± 0.70	0.70	
	0.9584	95.84			
	0.9512	95.12			
10	9.672	96.72	94.78 ± 2.05	2.20	95.22 ± 0.54
	9.264	92.64			
	9.498	94.98			
20	18.95	94.75	95.06 ± 0.85	0.90	
	18.88	94.41			
	19.206	96.03			

Table 2. Classification of raw material on the basis of defects in cocoa beans (ISO 1114, 1977).

Grade (G)	Mouldy	Slate-grey (colour altered)	Damaged Moth-eaten/ germinated/flat
G 1	≤3%	≤3%	≤3%
G 2	≤4%	≤8%	≤6%
SubG	≥4%	≥8%	≥6%

commonly encountered, OTA levels in cocoa beans artificially contaminated was found to be very high, ranging from 3.4 to 44.7 $\mu g\,kg^{-1}$, with a mean value of $22.9 \pm 3.6\,\mu g\,kg^{-1}$ (Table 3). A prior assessment survey of OTA contamination conducted on 298 samples of cocoa beans (10 kg each), collected from the commercial ports of Abidjan (151 samples) and San Pedro (147 samples) in Côte d'Ivoire, showed that average contamination did not exceed 2 $\mu g\,kg^{-1}$. The contamination levels were 1.30 ± 0.02 and $0.70 \pm 0.02\,\mu g\,kg^{-1}$ in Abidjan and San Pedro, respectively. The levels of OTA contamination observed in this survey mainly concerned raw materials selected and destined for export. To qualify for export, cocoa beans must meet the necessary quality standards displayed in Table 2. Only cocoa beans classified as Grade 1 or 2 qualify as export-grade. Thus, the implementation of these rigorous measures offers the most likely explanation for the low OTA levels encountered in both commercial ports. In addition, quantitative analysis for this survey was carried out according to Directive 2002/26/CE in the COFRAC-accredited laboratory of Lara Europe Analyses (Toulouse, France).

The contamination levels in our model were elevated 17.6–32.7-fold and sufficient for our study. Considering both parts of the cocoa bean, Table 3 indicates that the highest levels of OTA were detected in shells and at minor levels in nibs. For all samples analysed, the average OTA contamination of cocoa shells and nibs was 91.2 ± 17.1 and $6.9 \pm 1.7\,\mu g\,kg^{-1}$, representing 92.8 and 7.2%, respectively. The relative contribution of each part to the total contamination level, as shown in Figure 2, was calculated by dividing the concentration of OTA of each part, either shell (Cs) or nib (Cn), by the sum of both contamination levels (Cs + Cn), as follows:

$$\text{Shell OTA contamination}\,(\%) = [Cs/(Cs + Cn)] \times 100$$
$$\text{Nib OTA contamination}\,(\%) = [Cn/(Cs + Cn)] \times 100$$

These data are similar to previous observations reported by Amézqueta et al. (2005). As seen in Table 3, the disparity in the contamination levels observed between the data can be ascribed to the heterogeneity of the distribution of OTA

Table 3. OTA levels in cocoa beans, shells and nibs induced by the artificial contamination.

Samples	Unroasted beans ($\mu g\,kg^{-1}$)	Unroasted shells ($\mu g\,kg^{-1}$)	Unroasted nibs ($\mu g\,kg^{-1}$)
1	25.51	78.53	5.19
2	23.18	141	15.81
3	7.31	23.58	1.17
4	3.37	35.34	5.12
5	15.23	95.57	3.38
6	28.87	105.84	12.63
7	44.75	205.54	16.64
8	39.04	156.10	22.05
9	34.10	47.40	2.97
10	12.41	38.71	5.32
11	43.80	233.40	3.58
12	20.10	83.94	4.66
13	4.63	15.19	0.75
14	9.04	58.85	1.11
15	31.86	49.34	3.92
Average	22.88 ± 3.62	91.22 ± 17.10	6.95 ± 1.70

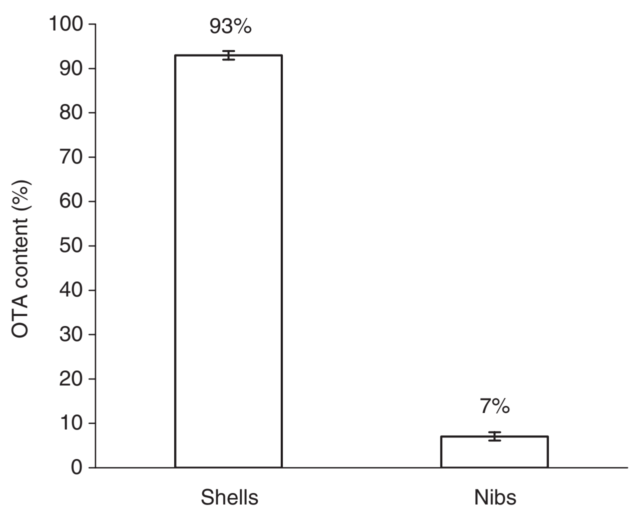

Figure 2. Percentage OTA content in each part of cocoa bean (shell and nib).

contamination on agricultural products. As previously reported by Da Glorial et al. (2004), this heterogeneous contamination can be compared with aflatoxin, another well-known mycotoxin, on agricultural products, including maize. In addition, the high contamination levels in our model confirm that improper harvest and post-harvest practices or poor storage conditions inducing a loss of physical integrity of cocoa pods are major factors in cocoa beans contamination by OTA-producing fungi.

Roasting process

The first step in industrial chocolate manufacturing is cocoa bean roasting. Under our experimental conditions, the roasting process was performed at 140°C for 30 min. The findings indicated that roasting has

Table 4. Percentage decrease in OTA contamination levels in cocoa beans, shell and nibs after the roasting process.

Samples	Roasted beans		Roasted shells		Roasted nibs	
	OTA level ($\mu g\,kg^{-1}$)	Decrease (%)	OTA level ($\mu g\,kg^{-1}$)	Decrease (%)	OTA level ($\mu g\,kg^{-1}$)	Decrease (%)
1	7.36	71.14	63.34	19.34	2.18	58
2	16.26	29.85	126	10.63	7.25	54.14
3	1.33	81.80	13.40	43.17	0.68	41.88
4	3.34	1	24.64	30.28	3.10	39.45
5	14.58	4.27	78.07	18.31	2.35	30.47
6	27.95	3.19	88.23	16.64	8.11	35.78
7	37.57	16.04	161.68	21.34	8.36	49.76
8	63.30	62.14	152.42	2.36	13.63	38.18
9	10.16	70.21	22.39	52.76	1.63	45.11
10	8.60	30.70	23.98	38.05	2.20	58.64
11	35.71	18.47	182.05	22	5.08	41.90
12	19.31	3.93	71.22	15.15	3.02	35.19
13	2.80	39.52	10.79	28.96	0.72	4
14	5.04	44.20	50.12	14.8	0.71	36
15	12.43	60.98	38.20	22.57	2.37	39.54
Average	17.72 ± 4.40	35.83 ± 7.24	73.77 ± 14.75	23.76 ± 3.37	$.10 \pm 0.96$	40.54 ± 3.41

a significant impact in terms of decreasing OTA content ($p < 0.01$); the decrease ranged from 23.7 to 40.5% (Table 4). This observation is in line with similar investigations carried out on other agricultural products, such as coffee (Scott et al. 1972).

In a previous study in our laboratory, coffee roasting was carried out above 200°C for 25 min; this resulted in a decrease of OTA content by >73% (Dano et al. 2009). Such conditions are considered to be extreme for cocoa processing. However, these data point to a close association between roasting parameters, i.e. temperature and time, and the extent of OTA destruction, as reported by others (Micco et al. 1989; Wilkens et al. 1999; Van der Stegen et al. 2001). Interestingly, the decrease in OTA content in nibs (40.54%) compared to shells (23.76%) was generally 1.7-fold higher (Table 4), which we interpret as the cocoa shell not being an obstacle to the diffusion of heat to the nib. Thus, the industrial process, which consists of roasting before shelling, is not a limiting factor. These roasting parameters could be revised to enhance the destruction of the OTA molecule without damaging the nib quality. In addition to its capacity to destroy OTA molecules, the roasting process has an extra advantage of facilitating the shelling process, thereby considerably reducing nib losses.

Shelling process

The second step in cocoa processing is shelling, which involves separating the shell from the nib. In chocolate production, our study showed that shelling represents a major step in the reduction of OTA levels. As mentioned earlier, we found that OTA contamination

Table 5. Percentage OTA removal after the shelling process.

Samples	Roasted beans ($\mu g\,kg^{-1}$)	Roasted nibs ($\mu g\,kg^{-1}$)	OTA removal (%)
1	7.36	2.18	70.38
2	16.26	7.25	55.41
3	1.33	0.68	48.87
4	3.34	3.10	100
5	14.58	2.35	83.88
6	27.95	8.11	70.98
7	37.57	8.36	77.75
8	63.30	13.63	62.32
9	10.16	1.63	83.96
10	8.60	2.20	74.42
11	35.71	5.08	85.77
12	19.31	3.02	84.30
13	2.80	0.72	74.20
14	5.04	0.71	85.90
15	12.43	2.37	80.93
Average	17.72 ± 4.40	4.10 ± 0.96	75.94 ± 3.38

in cocoa beans was mainly concentrated in the shell (92.8%), in contrast to the nib (7.2%). Consequently, shell removal resulted in a significant decrease in OTA levels by 48–100% ($p < 0.01$) (Table 5). Hence, shelling emerges as the most efficient way of detoxifying cocoa beans without damaging their quality by excessive heat. Similar observations were also recorded by Amézqueta et al. (2005), who demonstrated that shelling could induce a significant decrease in OTA levels in cocoa beans ranging from 50 to 95%. However, these authors also reported the results of previous studies carried out by CAOBISCO/ECA/FCC, which indicate that the decrease in OTA levels induced by shelling was lower (25–50%). This decrease in OTA levels was closely correlated with the method of shelling used by CAOBISCO/ECA/FCC. As in our study, Amézqueta et al. (2005) utilised a hand-made cocoa-shelling process and almost 100% of the shell was removed, whereas CAOBISCO/ECA/FCC used a mechanical procedure. Industrially, complete elimination (100%) of the shell is never achieved; in addition, there are technical difficulties in preventing shells and nibs coming into contact, which may result in nib contamination by OTA. As a consequence, these findings underline the importance of improving the mechanical shelling process in industry to prevent OTA occurrence in cocoa derivative products.

Additive addition

Another important industrial step in decreasing OTA content in the cocoa finished product is the addition of additives (sugar, milk, etc.) to the cocoa paste. In general, our study showed that the cocoa paste contains the lowest levels of OTA ($4.6 \pm 1.2\,\mu g\,kg^{-1}$) after undergoing the first three steps of the industrial treatment, i.e. roasting, shelling and crushing. Additive addition, the final operation in chocolate manufacture, is not regarded as a process leading to OTA degradation or removal, but rather a dilution of OTA, which represents a significant 50% decrease in OTA content ($51.47 \pm 5.90\%$) in the finished product (Table 6). However, the disparity in the decrease in OTA levels following additive addition is intriguing; a similar trend in reduction should have occurred, as all samples were treated under the same conditions. Many reasons can be proposed for this observation and the most likely explanation is related to variability in recovery. As mentioned previously, no recovery assay was performed on chocolate samples; only unroasted cocoa beans were subject to recovery testing, which proved satisfactory. However, it is possible that the obtained valid recovery ($95.22 \pm 0.54\%$) for unroasted beans is not applicable to the chocolate matrix. If so, the OTA levels would be relatively different. Chocolate is obtained after mixing the cocoa paste with sugar, cocoa butter and milk; thus, the resultant matrix is relatively different from cocoa paste and could contain compounds that may hamper extraction techniques or interfere with OTA antibodies in the IAC. Further investigations are necessary to verify the effect of chocolate matrix on OTA analysis. An alternative explanation could be a technical limitation. As indicated earlier, additive addition is viewed as a dilution process. Thus, to eliminate possible artefacts related to the manual mode, this step was performed mechanically using a chocolate machine (mixer), which should ensure complete homogenization of the chocolate paste. However, any heterogeneous distribution of OTA contamination in the sample material, despite the mixing, could result in such disparities.

Table 6. Decrease in OTA content of cocoa paste following additives addition.

Samples	Cocoa paste ($\mu g\,kg^{-1}$)	Chocolate ($\mu g\,kg^{-1}$)	Decrease (%)
1	2.84	1.57	44.72
2	6.04	2	66.88
3	0.58	0.53	8.62
4	1.78	0.77	56.74
5	3.20	1.78	44.38
6	19.10	3.16	83.46
7	9.54	4.03	57.76
8	11.14	6.69	40
9	2.27	1.26	44.50
10	1.02	0	100
11	3.83	1.55	59
12	4	1.48	62.80
13	0.72	0.53	26.30
14	1.66	1.23	25.90
15	2.12	1	55.23
Average	4.66 ± 1.31	1.84 ± 0.44	51.75 ± 5.90

Note: A value of $0\,\mu g\,kg^{-1}$ means that the OTA level is below the LOD.

Table 7. OTA levels in chocolate following the entire process of cocoa industrial treatment.

Samples	Unroasted beans ($\mu g\,kg^{-1}$)	Chocolate ($\mu g\,kg^{-1}$)	Decrease (%)
1	25.51	1.57	93.84
2	23.18	2	91.37
3	7.31	0.53	92.74
4	3.37	0.77	77.15
5	15.23	1.78	88.31
6	28.87	3.16	89.05
7	44.75	4.03	91
8	39.04	6.69	82.86
9	34.10	1.26	96.31
10	12.41	0	100
11	43.80	1.55	96.46
12	20.10	1.48	92.63
13	4.63	0.53	88.55
14	9.04	1.23	86.39
15	31.86	1	96.86
Average	22.88 ± 3.62	1.84 ± 0.41	90.90 ± 1.52

Note: A value of $0\,\mu g\,kg^{-1}$ means that the OTA level is below the LOD.

At the end of the chocolate-manufacturing process, the overall decrease in OTA amounted to 91%, when considering initial contamination levels (Table 7). Taken together, the observations summarized above provide strong evidence that the various industrial treatments induce a dramatic reduction in OTA levels from cocoa beans to its derivative products, such as chocolate. The cocoa industrial-processing steps, which have a real impact on OTA levels, can be classified into two major categories: those having direct action by destroying the chemical structure of OTA (roasting) and those exerting indirect action by removing the OTA molecule (shelling) or diluting the OTA concentration (additive addition).

Conclusions

Using our contamination model, we were able to show that industrial processing of cocoa has a significant impact in reducing OTA contamination levels. Industrial treatment is a detoxifying process, which reduces consumer exposure to OTA. Roasting, shelling and additive addition, three main steps in industrial treatment, significantly decreased OTA levels in cocoa finished products, thereby reducing OTA concentrations to safe levels. However, shelling emerged as the crucial step in cocoa detoxification. It is fundamental for manufacturers to ensure maximum separation of shells from nibs to guarantee a significant or complete removal of OTA for a safer cocoa product. Our results should be of interest to the regulatory authorities responsible for setting maximum limits for OTA in raw materials, particularly cocoa. Finally, harvest and post-harvest operations should be conducted under good agricultural practices to ensure an optimum initial physical quality of cocoa pods, thus preventing bean contamination by fungi. Future investigations will focus on cocoa pod quality as well as good agricultural practices pertaining to harvest/post-harvest and storage conditions.

Acknowledgements

This study was conducted within the framework of the program "Strategy for the prevention of ochratoxin A (OTA) contamination of coffee and cocoa in Côte d'Ivoire". The program was financially supported by the European Union (COM STABEX 99/1: DP OTA No. DP/IVC/2005/16), the organizations of coffee–cocoa network, including ARCC (Autorité de Régulation du Café Cacao), FRC (Fonds de Régulation et de Contrôle and BCC (Bourse du Café et Cacao). We thank the Ministry of Agriculture of Côte d'Ivoire for technical assistance.

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