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Traditional procedures of cocoa processing and occurrence of ochratoxin - A in the derived products

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ABSTRACT

Cocoa beans from several types of post harvest treatments were processed into cocoa butter, cocoa powder and chocolate spread using traditional processing techniques. OTA was analyzed in two hundred and sixteen (216) samples of cocoa and cocoa derived products using HPLC with fluorimetric detection. It was found that OTA content in cocoa nibs depended only on the state of cocoa pod and the time duration of cocoa pod-opening. Cocoa beans from damaged cocoa pods and pod-opening delayed by 10 days were the most contaminated by OTA. Neither roasting using fire wood, nor shelling by pounding in a mortar could reduce significantly the OTA content in cocoa beans. Apart from cocoa butter, the other cocoa derived products also had high OTA contents. Sixty seven (67%) of chocolate spread and cocoa powder samples were contaminated by OTA, and 50 % had contents superior to the limit defined by the European Union. Because of the fragile pellicle of cocoa beans, due to damaged pods and cocoa pod-opening delayed by 10 days, the contamination by OTA was no longer superficial and shelling does not longer contribute to reduce significantly the OTA content in beans. Like with modern procedures, traditional techniques of cocoa processing do not have significant influence on OTA content of derived products. Only the raw material (quality and state of cocoa pod) can influence the OTA content of cocoa derived products.

Keys words: cocoa, OTA, traditional techniques of cocoa processing, cocoa derived products

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INTRODUCTION

Ochratoxin A (OTA) is a mycotoxin produced mainly by fungi of Aspergillus and Penicillium genera. It is mainly produced by *Aspergillus carbonarius, Aspergillus niger, Aspergillus ochraceus* and *A. westerdijkiae* in tropical zones and by *Penicillium verrucosum or P. nordicum* in temperate zones [1, 36, 40]. In cocoa, only black *Aspergillus* of Nigri section (*A. carbonarius* and *A. niger* agg) are found [27].

The Ochratoxin - A has nephrotoxic[25], immunotoxic, teratogenic and carcinogenic effects [19, 21, 22]. Following research results obtained on animals, the International Agency for Research on Cancer (IARC) classified OTA as carcinogenic in humans (group 2B).

OTA occurs naturally in a wide variety of raw commodities and finished food products of plant origin such as cereals, coffee beans, cocoa beans, pulses, and dried fruits as well as cocoa derived products. Ochratoxin A presence has also been detected in lower amounts in meat, eggs and milk as a product of the carryover of animals feed with contaminated feedstuffs [2, 9, 12, 20, 29, 30, 32, 33, 37, 38, 39]. occurrence of OTA in cocoa powder and cocoa products was reported since 2000 by Engel [15]. During this decade, incidence and intake trends remained basically constant [32, 35]. The presence of OTA in cocoa is mainly related to cocoa bean shells and fat-free cocoa solids (cocoa powder) [3, 18].

Cocoa production in Cameroon is estimated at 200 000 tons per year. This production is from about 500 000 farmers and is distributed in three large zones of production. In Cameroon, the government does not control cocoa commercialization. Several actors intervene in cocoa commercialization; the middlemen buy at a derisory price (USD 1.6 per kg of cocoa) from farmers to resell at a higher price to exporters (about USD 3 per kg). In this case, farmers are the losers. National statistics have revealed a high level of poverty in the rural areas of Cameroon. Therefore, to improve the livelihood of farmers' particularly that of cocoa-farmers, the Government of Cameroon has adopted an agricultural policy that incites local consumption of cocoa. Following this, easy and applicable technologies were developed and disseminated to farmers, with the idea to process cocoa using local materials available in farmers' area.

Although several authors [9, 18] have studied the occurrence and OTA content of cocoa derived products obtained from modern and improved technics of cocoa processing, it is not yet the case of those resulting from the traditional technics.

The goal of this work was to assess the effects of traditional technics of cocoa processing on OTA content of some derived products of cocoa such as cocoa butter, cocoa powder, and chocolate spread.

EXPERIMENTAL SECTION

Cocoa

Cocoa (*Theobroma cacao* L.) was harvested in Kumba (Cameroon), during 2005, 2006 and 2007 cocoa seasons. Kumba, of the South West Region, is the biggest cocoa producing zone in Cameroon: accounting for more than 60 % of the national production, which stands at 180 000 tons annually. The type of cocoa grown in this region is the Amazonian. The climate is equatorial type with a total rainfall of 3000 mm year^{-1}.

Chemicals

Liquid chromatography grade methanol, acetonitrile and acetic acid were purchased from Carlo Erba (Carlo Erba Reagenti, Milan, Italy). Water was purified by distillation and passage through a Milli-Q system (Millipore, Bedford, MA, USA). Phosphate Buffer Solution (PBS) was used for the dilution step. Ochratoxin A certified standard solution (10.15 _ 0.14 mg/mL) was purchased from Biopure (Tulln, Austria). For clean-up step OCHRAPREP immunoaffinity column (IAC) was used (R-Biopharm Rhône Ltd, Glasgow, UK).

Post harvest treatments of cocoa and sampling

According to the type of fermentation (ie. heap or box), the physical appearance of the cocoa pod appearance (ie. damaged or not) or schedule for the splitting of the cocoa pods (immediately or delayed of 10 days), cocoa nibs went through four different types of post harvest treatments (table 1).

Table 1: Different post harvest treatments

Treatment	Cocoa pod appearance	Pod opening	Type of fermentation
T1	Intact	immediate	box
T2	Intentionally damaged	After 10 days	Heap
T3	Damaged on the tree	immediate	Heap
T4	Damaged on the tree	After 10 days	Heap

Fermented sun-dried cocoa from different technological treatments are used to fabricate derived products which are cocoa butter, cocoa powder and chocolate spread.

Butter extraction

Cocoa beans were placed in a locally made aluminum pot and roasted by stirring regularly for about 60 minutes under about 50°C. The roasted cocoa beans were introduced into a mortar and pounded lightly to facilitate removal of the husk by winnowing. Thereafter, the beans were finely ground into a paste with the aid of a mill. The paste obtained was mixed with water and cooked for several hours to extract the cocoa butter. The extracted cocoa butter was collected, washed using distilled water and filtered using a clean tissue.

Fabrication of chocolate spread and cocoa powder

After extracting the butter, cocoa paste was obtained by evaporating the water. The cocoa paste with reduce lipid contents was collected and mixed with sugar and refined oil to obtain chocolate spread which can be stored for about 3 months.

The paste of cocoa was dried to obtain cocoa cake. The dry cake was finely ground until the desired grade. Cocoa powder obtained can be consumed as hot drink with sugar during breakfast or as cold chocolate drink.

Sampling

Sampling was done at different steps of cocoa processing: fermented sun-dried cocoa (A), roasted cocoa (B), nibs (C), cocoa butter (D), cocoa powder (E) and chocolate (F). A total of seventy two (72) samples were analyzed during each cocoa season, given a total of 216 samples for the entire study period.

Titration of OTA

The dried cocoa bean samples were frozen at -80°C then ground. Fifty grams of ground beans or cocoa powder were extracted in 200 mL of solvent (acetonitrile/water, 60/40, v/v). Four millilitres of filtered extract were diluted in 44 mL of phosphate buffer. The mixture was purified on an immunoaffinity column (Ochraprep, R-Biopharm, Glasgow, UK). OTA was eluted by 3 mL of methanol and evaporated till dry in a nitrogen stream at 70°C. The residue was resuspended in 1 mL of mobile phase (water/acetonitrile/acetic acid, 51:48:1, v/v/v).

OTA was quantified on the extracts by HPLC with fluorimetric detection (Shimadzu LC-10 ADVP, Japan) [34]. The operating conditions were as follows: 100 µL injection loop, C18 reverse phase HPLC column, ODS particle size of 5 µm with an identical pre-column, thermostatically controlled at 35°C, isocratic flow of 1 $mL.min^{-1}$, an excitation wavelength of 333 nm and an emission wavelength of 460 nm. Contents were calculated from a calibration curve established from a standard (1 $\mu g.mL^{-1}$; ref PD 226 R-Biopharm, Glasgow, UK).

RESULTS AND DISCUSSION

Content of OTA in beans according to the type of post harvest treatment

Table 2 presents OTA contents of different fermented sun-dried cocoa according to the type of post harvest treatment. OTA contents were ranged between detection limit (dl < 0.03 $ng.g^{-1}$) and 0.15 $ng.g^{-1}$ when beans were provided from not damaged cocoa pods T1; which remains less than 2 $ng.g^{-1}$ which is the tolerable set defined for cocoa beans (European Commission, 2006). Low OTA content of cocoa was also obtained in beans from cocoa pods damaged on the tree, and pod opening immediately (T3).

However, when pods were not intact and cocoa pod - opening was deferred (T2, T4), cocoa beans resulting from those treatments were the most contaminated by OTA (table 2). Maximum content of 20.75 $ng.g^{-1}$ (value which is higher than tolerable set) was observed with treatment T4.

Therefore, the contamination by OTA did not depend on the type of fermentation (box or heap). The presence of OTA during cocoa post harvest depended on the state of cocoa pod and the deadline of cocoa pod - opening.

These results are in accordance with those of Bastide and al. [6] who showed that the presence of OTA during post harvest treatment of cocoa depends on the cocoa pod state. According to the same authors, bean OTA content depended on the phytosanitary condition of pods, with 4.2 $ng.g^{-1}$ for pods damaged by insects, 19.8 ng/g for wounded pods, 7.2 $ng.g^{-1}$ for rotten cocoa pods and 3.4 $ng.g^{-1}$ for mummified pods. Studies made by Suàrez-Quiroz and al., [42] have shown that it was possible to compare high contents in OTA to the presence of d'*Aspergillus carbonarius*. Contamination by *Aspergillus carbonarius* was mainly found in damaged cocoa pods with deferred cocoa pod - opening. When cocoa pods were partially covered, cocoa beans were in direct contact with air and soil, which led to high ochratoxinogenics activities [28].

OTA content of beans according to cocoa season year

Our results have shown that, treatments T2 and T4 are generally those that lead to high contamination of cocoa by OTA. It was necessary to observe if the year of cocoa season could have an effect on this contamination.

OTA contents vary. Table 3 showed that irrespectively of the cocoa season, the maximum contamination was always obtained with beans from tree damaged cocoa pods and cocoa pod - opening delayed by 10 days (T4). Only the maximum content varies according to year. The 2005 cocoa season was the most polluted with a content of 20.75 $ng.g^{-1}$. Only half (10 $ng.g^{-1}$) of this content was obtained in 2006 and 2007 cocoa seasons.

This study was done in Kumba area which is an agro-ecological zone with a monomodal rainfall. The mean annual rainfall is 3000 mm. This implicates a high humidity (~90 %). The difference in OTA content obtained could be due to other environmental factors that are difficult to control such as the level of air pollution by ochratoxinogenic fungi, speed of wind, etc.

Bastide and al., [6] have also shown that OTA contents could vary with the cocoa season.

Table 2: OTA contents of cocoa beans and different cocoa derived products

Type of treatment	Sampling	OTA content: ng.g^{-1} (2005 season)	OTA content: ng.g^{-1} (2006 season)	OTA content: ng.g^{-1} (2007 season)
T1	Cocoa beans	0.15 ± 0.01	nd	0.6 ± 0.00
	Roasted Cocoa	nd	0.03 ± 0.01	nd
	Nibs	nd	0.02 ± 0.00	0.01 ± 0.00
	butter	nd	nd	nd
	Cocoa powder	0.04 ± 0.01	nd	0.03 ± 0.02
	Chocolate spread	nd	0.01 ± 0.01	0.03 ± 0.00
T2	Cocoa beans	0.16 ± 0.02	1,14 ± 0.34	5.34 ± 0.21
	Roasted Cocoa	0.08 ± 0.00	0.92 ± 0.02	5.12 ± 0.82
	Nibs	nd	nd	4.08 ± 0.11
	butter	0.01 ± 0.00	nd	0.02 ± 0.00
	Cocoa powder	nd	0.01 ± 0.01	1.55 ± 0.07
	Chocolate spread	nd	nd	1.95 ± 0.36
T3	Cocoa beans	0.04 ± 0.00	0.25 ± 0.01	0.05 ± 0.00
	Roasted Cocoa	0.05 ± 0.04	0.5 ± 0.21	0.03 ± 0.03
	Nibs	nd	nd	0.02 ± 0.02
	butter	0.02 ± 0.00	0.01 ± 0.01	0.01 ± 0.01
	Cocoa powder	0.03 ± 0.01	0.13 ± 0.00	nd
	Chocolate spread	nd	0.03 ± 0.02	nd
T4	Cocoa beans	20.75 ± 1.22	10.0 ± 0.22	10.0 ± 1.22
	Roasted Cocoa	19.86 ± 0.89	10.11 ± 1.04	9.80 ± 1.01
	Nibs	18.19 ± 1.07	9.45 ± 0.99	8.22 ± 1.33
	butter	1.88 ± 0.32	1.10 ± 0.44	0.93 ± 0.61
	Cocoa powder	16.15 ± 1.11	7.22 ± 0.77	6.99 ± 0.33
	Chocolate spread	17.00 ± 1.58	8.89 ± 0.69	7.33 ± 1.01

Table 3: Influence of the cocoa season year on beans OTA content

Type of treatment	**OTA content: ng.g^{-1}**		
	2005 season	**2006 season**	**2007 season**
T2	0.16 ± 0.02	1.14 ± 0.34	5.34 ± 0.21
T4	**20.75 ± 1.22**	**10.0 ± 0.22**	**10.0 ± 1.22**

TECHNOLOGICAL TREATMENT OF COCOA BEANS AND OTA CONTENT

OTA content in roasted beans

The OTA content of roasted beans from undamaged cocoa pods ranged between the nd and 0.03 ± 0.01 ng.g^{-1} (value inferior to the tolerable limit for cocoa beans). In addition, significant differences in OTA content were not observed with roasted beans obtained from pods that were damaged by birds or cutlasses during harvesting.

However, late cocoa pod-opening (delayed by 10 days) of the same beans contributed to obtain fermented sun-dried cocoa with maximum content of OTA 5.34 ± 0.21ng.g^{-1} for T2 and 20.75 ± 1.22 ng.g^{-1} for T4. After roasting using fire wood for almost an hour, OTA contents are 5.12 ± 0.82 ng.g^{-1} and 19.86 ± 0.89 ng.g^{-1} respectively. Therefore, roasting did not have an impact on beans OTA content.

OTA conserves its stability during most of the steps of foods processing such as cooking, washing and fermentation [5]. Boudra and al. [8] have shown that OTA is resistant to heat and a maximum of 20 % of OTA in wheat is decomposed by dry heat at 100 °C for 160 minutes or at 150 °C for 32 minutes. Suàrez-Quiroz and al., [43] observed a reduction of OTA content in coffee after thermal treatment. These authors explained the reduction by a possible complexation with other molecules of the milieu. Mounjouenpou and al. [26] also noted a reduction of OTA content with heating. This reduction depends on the pH of the milieu with the largest reduction observed at pH>7 (alkaline medium) and at 85 °C. During cocoa roasting, the final temperature of beans reaches 100 to120°C for a period of 15 to 70 minutes [4, 23, 31].In these conditions, it is not expected that roasting reduces OTA at higher levels because a significant degradation can only be possible at 180-200°C [16, 43]. Traditional procedures of cocoa beans roasting applied in rural zones that consist to roast in a pot does not therefore present any impact on OTA content of the product because the temperature of beans remains less than 100°C.

OTA content of nibs

Roasted cocoa beans was shelled by pounding in a mortar and winnowing to obtain the nibs. When pod-opening was immediate, (T1 and T3), OTA content in nibs remained less than the limit of detection (<0.03 ng.g^{-1}).

However, when nibs result from a treatment where cocoa pod is damaged, and pod-opening was deferred, almost all the OTA of roasted beans was found in nibs. When cocoa pods are intentionally damaged, OTA passes from 5.12 ± 0.82 ng.g^{-1} in roasted beans to 4.08 ± 0.11 ng.g^{-1} in nibs during the 2007 season. For cocoa pods naturally injured, values are respectively 19.86 ± 0.89 ng.g^{-1} and 18.19 ± 1.07 ng.g^{-1} in 2005.

Generally, literature reveals a reduction of OTA content in cocoa nibs due to shelling. Gilmour, and Lindblom [18] analyzed OTA in 15 samples pairs of cocoa shell and bean. Results showed that a mean of 48 % (ranging from 25 to 72 %) of OTA present in cocoa bean was eliminated together with the shell. In a study were cocoa shell were eliminated manually, Amèzqueta and al., [43] observed a reduction of OTA content (>95 %) in 14 out of 22 samples, of 65 to 95 % in 6 out of 22 samples and of less than 50 % in only one of the samples.

Contrary to the literature, our results showed that shelling does not necessary reduce OTA content in cocoa nibs. With damaged cocoa pods and deferred pod-opening of 10 days, cocoa beans start to germinate. The protector pellicle of almonds is not longer entire as it breaks out in order to allow the young plant grow. With fermentation and drying, beans are more fragile. It crumbs with a least friction between fingers. Ochratoxinogenic fungi and OTA cross the shell and are mostly found at the level of 'carbohydrate' substrate which almonds represent. The localization of OTA depends therefore of the cocoa beans state (integrity of the shell, bean of good quality).

OCCURRENCE OF OTA IN DERIVED COCOA PRODUCTS

Titration of OTA in cocoa butter

Samples of cocoa butter extracted are less contaminated by OTA: 25 % of butter samples have an OTA content superior to the limit of detection (0.03 ng.g^{-1}) and 67 % of butter samples have an OTA content superior or equal to European tolerable limit, defined for derived cocoa products which is 1 ng.g^{-1}. The maximum content titrated was 1.88 ± 0.32 ng.g^{-1}. This content is superior to the norm. Butter samples contaminated by OTA are exclusively extracted from nibs obtained in damages cocoa pods and deferred pod-opening (T4).

OTA is soluble in polar organic solvents, in diluted solution of sodium bicarbonate, moderately soluble in water [41]. Therefore, during the cooking of cocoa paste, butter is liberated from oleiferous cells and floats on the surface. The biggest part of OTA contained in the sample will therefore remain in the solid fraction; only a very little part could be found in the lipid part.

Titration of OTA in cocoa powder

Cocoa powder produce have reduce lipid contents. A total of 36 samples of cocoa powder were produced and analyzed at HPLC. Sixty seven percent (67 %) of these samples are contaminated by OTA (content superior or equal to 0.03 ng.g^{-1}) and 50 % of positive samples have an OTA content superior to the tolerable set defined for cocoa derived products (1 ng.g^{-1}). The maximum content is 10.15 ng.g^{-1} during the 2005 cocoa season.

With the cooking, a light drop of OTA content were observed: from 18.19 ng.g^{-1} in nibs to 16.15 ng.g^{-1} in cocoa powder providing from treatment T4 of the 2005 cocoa season. Generally, an average reduction of 10 % was observed in positive samples because of cooking.

Cocoa powder constitutes one of the most contaminated fractions by OTA [7, 13, 18, 24]. Vecchio & Finoli [44] showed that 82 % of cocoa powder commercialized in Italy was contaminated by OTA. During cooking, OTA remains in the solid phases. During this thermal treatment, it is possible that a complex is made by OTA and other carbon molecules present in the milieu [43]: this explains the reduction in OTA content of about 10 % observed.

Titration of OTA in chocolate

Like in the cocoa powder, chocolate is one of the most contaminated cocoa derived products by OTA. Sixty seven (67 %) of chocolate samples are also contaminated by OTA, and 50 % have contents superior to the norm defined by the European Union. The maximum content titrated is 16.15 ng.g^{-1} observed on treatment T4 of the 2005 cocoa season.

A large preponderance of OTA in chocolate samples is revealed in the literature. It is the case of Burdaspal and Legarda in 2003 [11] who evaluated the occurrence of OTA in 296 samples of different types of chocolate in Spain and 15 other countries. OTA was detected in all the samples, except one (99.7 % of positive samples). Similar study was done by many other authors in Italy, German and other countries with the same results (large OTA content of chocolate sample) [24, 32].

INFUENCE OF THE PROCEDURES OF TRANSFORMATION ON THE LEVEL OF OTA IN PRODUCTS

Thirty six (36) samples of cocoa beans coming from several types of post harvest treatments were transformed by traditional techniques into cocoa butter, cocoa powder and chocolate paste. This was done in order to determine the influence of the procedure of transformation on OTA content of process products. Among 36 samples of cocoa beans analyzed, 12 samples had OTA contents superior to the threshold tolerable for cocoa beans which is 2 $ng.g^{-1}$. Only these beans are taking into consideration to study the impact of procedures on OTA content. Table 4 presents the mean contents of contaminated beans and derived products.

Table 4. Mean content of OTA in contaminated nibs and derived products

Samples	OTA content ($ng.g^{-1}$)	Mean content ($ng.g^{-1}$)
Cocoa beans	5.34 20.75 10.0 10.0	11.52
Roasted Cocoa	5.12 19.86 10.11 10.11	11.30
Nibs	4.08 18.19 9.45 8.22	9.98
Cocoa Butter	0.02 1.88 1.10 0.93	0.98
Cocoa powder	1.55 16.15 7.22 6.99	7.97
Chocolate spread	1.95 17.00 8.89 7.33	8.79

Figure 1 gives the influence of traditional procedures on OTA content in transformed products. This figure shows that derived cocoa products conserve a high contamination by OTA. Neither shelling nor traditional procedures have contributed to reduce the OTA content of products. Only cocoa butter is lightly contaminated (0.98 $ng.g^{-1}$). Cocoa powder and chocolate spread have a proximally the same OTA mean content (about 8 $ng.g^{-1}$).

Similar works were done by Dembele and al. in 2009 [14] in Ivory Coast. This study was made on 16 samples of cocoa with more important levels of OTA (contents ranged between 3.37 and 46.15$\mu g.kg^{-1}$). These samples were transformed using modern techniques into derived cocoa products (cocoa butter, chocolate…). In average, about 70 % of OTA was taken out with the shell fraction and processed products obtained were lightly contaminated by OTA. Chocolates contained 1.86 $\mu g.kg^{-1}$in averages and butter was free from OTA.

Because of the fragility of bean pellicle due to injured pods and deferred pod-opening, contamination by OTA is not longer superficial and ‘shelling does not longer contribute to reduce significantly OTA content in beans. This therefore explains important OTA contents of derived cocoa products.

Traditional procedures of cocoa processing do not have an influence on OTA content in derived products.

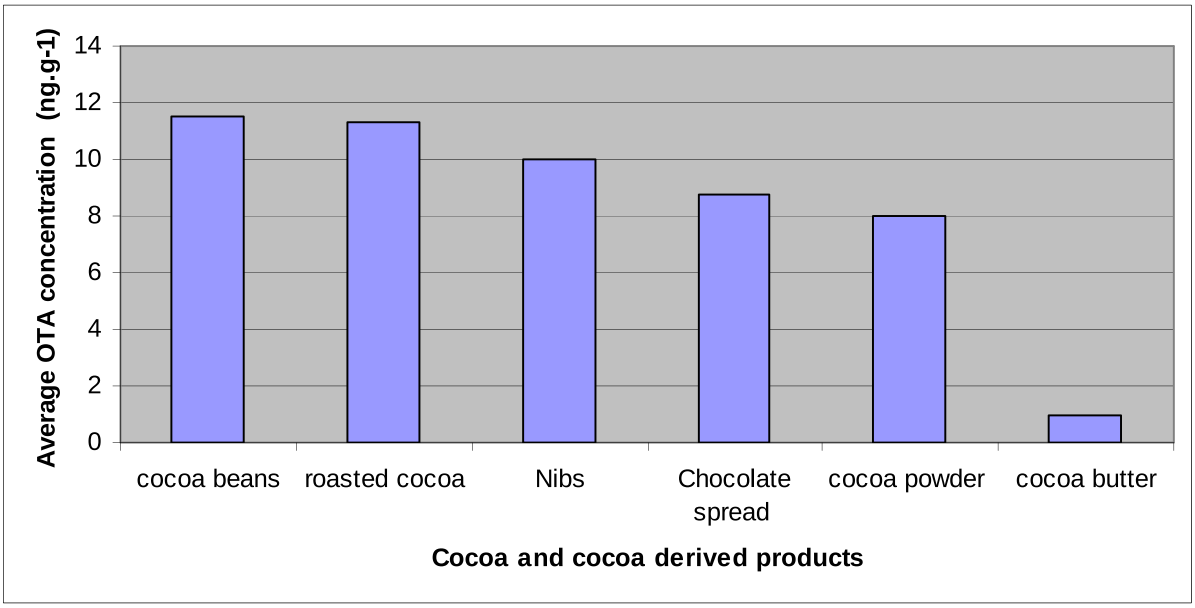

Figure 1: Influence of traditional procedures on Ochratoxin A (OTA) content in transformed products…

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