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FOOD SAFETY AND QUALITY - RESEARCH PAPER

Occurrence of ochratoxin A in cocoa beans and bean-to-bar chocolates

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Abstract
The growing health consciousness of consumers has led to an increase in the consumption of artisanal chocolates, mainly due to their recognized health benefits. However, processing steps such as fermentation and drying of cocoa beans can favor the growth of ochratoxigenic fungi. This study aimed to assess the occurrence of ochratoxin A (OTA) in cocoa beans (purchased from e-commerce and post-harvest processing) and bean-to-bar chocolates sold in Brazil. An HPLC-FLD method was validated, with recovery values between 84 and 97% and limits of detection and quantification of 0.04 and 0.01 µg/kg, respectively. OTA was detected in 30% of the cocoa bean samples studied (n = 43), with values ranging from < 0.04 to 1.18 µg/kg. Regarding the bean-to-bar chocolates (n = 62), the OTA concentrations ranged from < 0.04 to 1.11 µg/kg, with a prevalence in semi-sweet and dark chocolates. Despite representing a growing market, to the best of our knowledge, this is the first study to report OTA concentrations in bean-to-bar chocolates and Brazilian cocoa beans used to produce this type of chocolate.

Keywords Artisanal chocolate · Mycotoxin · Food safety · HPLC-FLD

Introduction

Bean-to-bar chocolate emerged in the 1990s in the United States. It is made from dried and fermented cocoa beans and can contain sugar, milk, fruits and nuts, and spices. The cocoa beans used for its production are traceable and controlled during all the processing stages [1]. However, cocoa beans may be exposed to ochratoxigenic fungi during harvesting, fermentation, drying, and storage [2]. Among the species that produce ochratoxin A, the literature cites a diversity of species of the genus *Aspergillus*, responsible for the contamination of ochratoxin A. The species isolated from cocoa beans are *A. carbonarius, A. niger, A. ochraceus, A. meleus*, and *A. westerdjikiae* [3,4], which can remain in the chocolates produced with contaminated beans [5,6].

The consumption of OTA-contaminated food can cause harmful effects on public health, as OTA is classified by the International Agency for Research on Cancer (IARC) in group 2B as a possible carcinogen [7]. Regulatory agencies in Brazil and Europe have set limits of 10 µg/kg and 5 µg/kg for cocoa products and chocolates, respectively [8] and 3 µg/kg for cocoa powder (EU, [9]). In addition, the FAO/WHO expert committee on food additives (JECFA) has set a provisional tolerable weekly intake (PTWI) of 112 ng/kg bw for OTA [10].

The determination of OTA in food is carried out using solvent extraction techniques with methanol and acetonitrile, solid phase extraction (SPE) with selective solvents, as well as the use of immunoaffinity columns (IAC) [11,12], SPE with non-selective solvents [13,14], and methods using molecularly imprinted polymers (MIPs), as described by Pichon & Combes [15]. OTA quantification is generally performed using liquid chromatography with fluorescence

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Research highlights
- OTA was positive in 30% of cocoa bean samples.
- PTWI values of 7.3 and 1.8% were estimated for children and adults, respectively.
- OTA in chocolates was found within Brazilian and European regulations.

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detector (HPLC-FLD) [3,16], gas chromatography with electron capture detector (GC-ECD) [17], capillary electrophoresis with laser-induced fluorescence detector (CE-LIF) [18] and high-performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS) [19,20].

Despite representing an expanding market [1,21], there is still a gap in knowledge about the occurrence of OTA in cocoa beans and bean-to-bar chocolates. Thus, this study aimed to assess the occurrence of ochratoxin A in the bean-to-bar chocolate production chain. For that, 105 samples were studied, comprising cocoa beans destined for the production of artisanal chocolates, fermented and dried cocoa beans from different processing stages, and bean-to-bar chocolates available on e-commerce in Brazil. In addition, the estimated intake of OTA for adults and children through the consumption of these foods was determined.

Material and methods

Sampling

Cocoa beans were purchased from the main producing regions in Brazil (Amazonas, Bahia, Espirito Santo and Para), comprising e-commerce samples (n = 21) and fermented and dried samples from post-harvest processing on farms located in Arataca-BA (n = 14) and Mocajuba-PA (n = 8), in quantities ranging from 80 g to 2 kg of sample. The bean-to-bar chocolate samples (n = 62) produced with cocoa beans of traceable origin from various regions of Brazil (Amazonas, Amapa, Bahia, Espirito Santo, Para and Rondonia) were purchased via e-commerce. The chocolate bars weighed between 25 and 100 g and some samples (n = 11) contained ingredients such as hazelnuts, bananas, coconut milk, goat's milk, cashew nuts, coffee, peanuts, dates, plant-based milk, and red fruits. The bean-to-bar chocolate samples were classified according to the information on the label as white chocolate, milk chocolate (40 to 56% cocoa solids), semi-sweet chocolate (40 to 68% cocoa solids), and dark chocolate (70 to 100% cocoa solids). The composition of all samples is shown in Tables 1 and 2.

The samples were stored in sterile packaging and kept at low temperatures in a dark environment until analysis.

Determination of ochratoxin A in cocoa beans and bean-to-bar chocolate

OTA extraction was carried out using the method proposed by Copetii et al. (2010), with modifications. Ten grams of sample (chocolate bars and cocoa beans) were ground in a mill (IKA, Staufen, Germany) and heated in a water bath at 65 ºC for 10 min. Then, 200 mL of 3% (1:1 v/v) methanol: sodium bicarbonate solution was added, and the mixture was homogenized for 10 min at 10,000 rpm. The mixture was filtered through qualitative paper and a glass microfiber filter (Whatman, Maidstone, UK), and 20 mL of the filtered extract was diluted in 20 mL of phosphate-buffered saline with 0.01% Tween 20 (Sigma-Aldrich, St. Louis, USA). The resulting solution was passed through an immunoaffinity column (Ochraprep, R-Biopharm, Darmstadt, Germany) at a flow rate of 2–3 mL/min. The column was washed with 20 mL of purified water (Milli-Q, Merck-Millipore, Darmstadt, Germany) and the OTA was eluted with 4 mL (8 × 500 µL) of methanol: acetic acid solution (98:02, v/v) in an amber vial. The contents were evaporated at 40 ºC under a stream of nitrogen and resuspended in 0.4 mL of mobile phase.

OTA was determined using high-performance liquid chromatography (Agilent Infinity 1260, Santa Clara, USA), with a fluorescence detector at 333 nm (λex) and 477 nm (λem), using a C18 column 250 mm × 4.6 mm and 5 µm particle size (Shimadzu Shim-pack, Tokyo, Japan). The mobile phase consisted of methanol: acetonitrile: water: acetic acid (35:30:34:01, v/v/v/v), at a flow rate of 1 mL/min. Quantification was carried out using a six-point analytical curve (µg/mL), made from an OTA standard (Sigma-Aldrich, St. Louis, USA). The injection volume was 100 µL for both the analytical curve and sample extracts.

Table 1 Composition of cocoa beans samples

Origin of cocoa beans	
Brazilian E-commerce	Amazonas (AM): Borba (*n* = 2), Manicore (*n* = 5), Nova Olinda (*n* = 1)
	Bahia (BA): Ibirapitanga (*n* = 2), Itabuna (*n* = 2), Itajuípe (*n* = 1), Porto Seguro (*n* = 2), not identified (*n* = 3)
	Espirito Santo (ES): Linhares (*n* = 2)
	Para (PA): Novo Repartimento (*n* = 1)
Post-harvest processing	Arataca (BA, *n* = 14): Fermentation day 1 (*n* = 2), Fermentation day 2 (*n* = 2), Fermentation day 3 (*n* = 2), Fermentation day 4 (*n* = 2), Fermentation day 5 (*n* = 2), Fermentation day 6 (*n* = 2), Drying (*n* = 3)
	Mocajuba (PA, *n* = 8): Fermentation day 1 (*n* = 1), Fermentation day 2 (*n* = 1), Fermentation day 3 (*n* = 1), Fermentation day 4 (*n* = 2), Fermentation day 5 (*n* = 1), Fermentation day 6 (*n* = 1), Drying (*n* = 1)

Table 2 Composition and main ingredients of bean-to-bar chocolate samples

Chocolate sample	Cocoa beans origin	Composition	
		Main ingredients	Cocoa mass (%)
AM1	Amazonas (AM, $n = 10$)	Blank chocolate	-
AM2		Chocolate and coconut milk	40
AM3		Milk chocolate	40
AM4		Semi-sweet chocolate, cocoa nibs	65
AM5		Dark chocolate	70
AM6		Dark chocolate	70
AM7		Dark chocolate	70
AM8		Dark chocolate	70
AM9		Dark chocolate	81
AM10		Dark chocolate	100
AP1	Amapa (AP, $n = 2$)	Semi-sweet chocolate	40
AP2		Dark chocolate, cocoa nibs	70
BA1	Bahia (BA, $n = 32$)	Milk chocolate	40
BA2		Milk chocolate	42
BA3		Semi-sweet chocolate and nutmeg	42
BA4		Semi-sweet chocolate	42
BA5		Milk chocolate	45
BA6		Semi-sweet chocolate	45
BA7		Semi-sweet chocolate	50
BA8		Milk chocolate	50
BA9		Milk chocolate	52
BA10		Chocolate and vegetal milk	54
BA11		Chocolate, coconut milk and cashew nuts	55
BA12		Chocolate and goat milk	56
BA13		Milk chocolate	56
BA14		Semi-sweet chocolate	56
BA15		Semi-sweet chocolate	60
BA16		Semi-sweet chocolate	65
BA17		Semi-sweet chocolate	67
BA18		Semi-sweet chocolate	68
BA19		Dark chocolate	70
BA20		Dark chocolate	70
BA21		Dark chocolate	70
BA22		Dark chocolate	70
BA23		Dark chocolate and xylitol	70
BA24		Dark chocolate and dates	70
BA25		Dark chocolate	70
BA26		Dark chocolate	70
BA27		Dark chocolate	70
BA28		Dark chocolate	70
BA29		Dark chocolate	70
BA30		Dark chocolate	75
BA31		Dark chocolate and coffee	75
BA32		Dark chocolate	100

Table 2 (continued)

Chocolate sample	Cocoa beans origin	Composition	
		Main ingredients	Cocoa mass (%)
ES1	Espirito Santo (ES, $n=12$)	Milk chocolate	40
ES2		Milk chocolate	40
ES3		Milk chocolate	40
ES4		Milk chocolate	40
ES5		Semi-sweet chocolate	40
ES6		Dark chocolate	70
ES7		Dark chocolate	70
ES8		Dark chocolate	70
ES9		Dark chocolate and dried banana	77
ES10		Dark chocolate	77
ES11		Dark chocolate and peanut	78
ES12		Dark chocolate	80
PA1	Para (PA, $n=4$)	Blank chocolate and berries	-
PA2		Semi-sweet chocolate	50
PA3		Dark chocolate	70
PA4		Dark chocolate	100
RO1	Rondonia (RO, $n=2$)	Milk chocolate	55
RO2		Dark chocolate	70

Quality control and statistical analysis

The method was validated for accuracy (spiked experiments), limit of detection (LOD), limit of quantification (LOQ), linearity, and precision. The limits of detection (LOD) and quantification (LOQ) were determined using the equations: LOD = 0 + t.s and LOQ = 10.s, where s = standard deviation, t = tstudent for 99% confidence [22]. Recovery tests were carried out at three levels (1.74, 4.36, and 10.89 μg/kg), covering the range of the calibration curve, and precision was assessed by the coefficient of variation of seven analytical repetitions for each sample (AOAC, [23]). All results were analyzed by one-way ANOVA and the Tukey test at a 95% confidence level (Statistica—StatSoft, Tulsa, EUA).

Exposure assessment

For the evaluation of the estimated exposure to OTA from the consumption of bean-to-bar chocolates, the daily intake of 15.75 g of chocolate [21], the standard body weight (bw) of 60 kg for adults and 15 kg for children, and the highest OTA level found in the samples (1.11 μg/kg) were considered. The results of the estimated OTA intake were compared to the tolerable weekly intake of 112 ng/kg bw [10].

Results and discussion

The extraction and quantification methods proved to be satisfactory, with a complete separation of OTA into a well-defined peak and a retention time of 10.76 min (Fig. 1). The limits of detection and quantification were 0.04 and 0.10 μg/kg, respectively, and the analytical curves were considered linear ($r^2 > 0.9999$). Recovery results were 84 to 95% and 86 to 97% for cocoa beans and chocolates, respectively, which are in agreement with AOAC ([23]) recommendations (60–115%). Coefficients of variation of between 13 and 15% were observed for cocoa beans and chocolate samples, respectively. This variation is also in agreement with proposed by AOAC ([23]) and INMETRO [22].

Presence of ochratoxin A in cocoa beans from e-commerce

The results (mean and range) of ochratoxin A concentration in the cocoa bean samples are shown in Table 3.

As can be seen in Table 3, 48% of the samples purchased from e-commerce were positive for OTA, i.e., this mycotoxin was found at levels above the LOD. Nonetheless, all samples were found at safe levels according to the maximum limits established for cocoa beans and cocoa powder by the Brazilian [8] and European (EU, 2023) legislations: 10 μg/kg and 3 μg/kg, respectively.

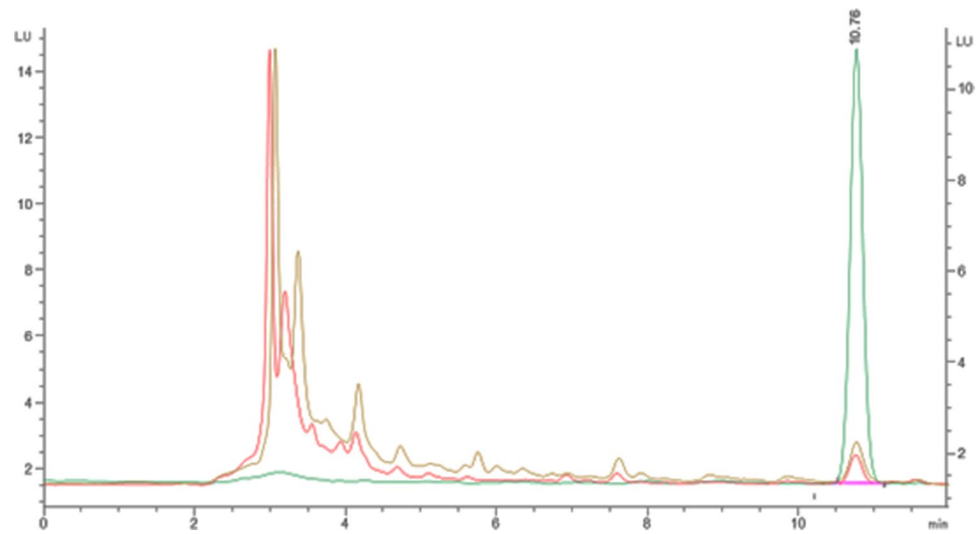

Fig. 1 Chromatogram of the separation of ochratoxin A. Cocoa beans (orange), chocolate (red), and standard 11 µg/kg (green)

Table 3 OTA concentrations (mean and range) of cocoa beans from different Brazilian regions

Origin	Mesoregion	City	Positive for OTA	Mean (range) (µg/kg)
E-commerce ($n=21$)	AM ($n=8$)	Borba ($n=2$)	1	0.25[a] (ND–0.50)
		Manicore ($n=5$)	3	0.30[a] (ND–0.64)
		Nova Olinda ($n=1$)	0	ND
	BA ($n=10$)	Ibirapitanga ($n=2$)	2	0.76[a] (0.34–1.18)
		Itabuna ($n=2$)	2	0.58[a] (0.23–0.93)
		Itajuipe ($n=1$)	1	0.91[a]
		Not identified ($n=3$)	0	ND (ND–ND)
		Porto Seguro ($n=2$)	1	0.33[a] (ND–0.66)
	ES ($n=2$)	Linhares ($n=2$)	0	ND (ND–ND)
	PA ($n=1$)	Novo Repartimento ($n=1$)	0	ND
Post-harvest processing ($n=22$)	BA ($n=14$)	Arataca ($n=14$)	2*	0.16[a] (ND–1.17)
	PA ($n=8$)	Mocajuba ($n=8$)	0	ND (ND–ND)

ND = not detected, OTA < 0.04 µg/kg

*Drying stage

[a]Different letters in samples from the same origin indicate significant differences between cocoa beans from different Brazilian regions (one-way ANOVA + Tukey test, $p < 0.05$)

Cocoa beans from the regions of ES (Linhares), and PA (Novo Repartimento) presented no OTA contamination. In turn, the positive samples came from AM (50%) and BA (60%) regions, which had the highest OTA concentrations: 0.64 µg/kg (Manicore), 0.66 µg/kg (Porto Seguro), 0.93 µg/kg (Itabuna), and 1.18 µg/kg (Ibirapitanga). These results are in line with the findings of Pires et al. [4], who reported mean OTA concentrations of 0.67 µg/kg in 25% of the cocoa bean samples studied (Bahia region) and Kulahi & Kabak [24], who found between 0.22 and 1.08 µg/kg OTA in 25 samples from Turkey. Higher OTA concentrations were reported by Calderón et al. [25] when compared to the present study, with levels between 2.32 and 4.77 µg/kg for 22 samples of cocoa beans from Chile. The presence of OTA may be associated with the drying stage of cocoa beans, which leads to a decrease in water activity (Aw) and the restriction of the growth of bacteria and yeasts that compete with toxigenic fungi. In our study, Aw ranged from 0.976 (fermented cocoa bean) to 0.622 (fermented and dried cocoa bean).

Presence of ochratoxin A in cocoa beans from post-harvest processing

In this study, cocoa beans from post-harvest processing for the production of bean-to-bar chocolates were obtained from two cocoa farms and subjected to the fermentation (6 days) and drying stages, totaling 22 samples. The occurrence of

OTA was detected in two samples (1.08 and 1.17 µg/kg), both from the drying stage in the Arataca farm. Studies on cocoa beans destined for conventional chocolate production have reported higher OTA concentrations. Copetti et al. [3] reported values of up to 5.54 and 4.64 µg/kg in cocoa beans from the drying and storage stages. Dano et al. [26] studied samples from the central, western, and southeastern regions of Côte d'Ivoire, and reported the occurrence of OTA in 29 of the 51 samples studied, with values of 0.27 and 0.57 µg/kg for the samples from the fermentation and drying stages, respectively, with the highest levels between the 4th and 7th days of fermentation. In turn, Kedjebo et al. [27] found levels of 3.63 and 2.21 µg/kg (4th and 7th days, respectively) in cocoa beans from farms in Akoupe, Côte d'Ivoire, while Delgado-Ospina et al. [28] found 188.9 µg/kg (6th day) in cocoa beans from a farm in Valle de Cauca, Colombia, with low mold counts (1.0 ± 0.1 log CFU/g). The authors reported that the presence of OTA in the samples from the fermentation stage is evident when favorable conditions are met, such as water activity between 0.935 and 0.965 and temperatures between 25 and 32 ºC.

Presence of ochratoxin A in bean-to-bar chocolates

The results of ochratoxin A concentrations (mean and range) of the bean-to-bar chocolate samples are shown in Table 4.

As shown in Table 4, 23% of the samples were positive for OTA, and the highest levels were observed for the samples from BA (1.11 µg/kg) and ES (0.48 µg/kg) in semi-sweet and dark chocolates, respectively. OTA was not detected (LOD = 0.04 µg/kg) in the samples from the AP and RO regions. Concerning the different chocolate samples, the highest mean levels were found for semi-sweet bean-to-bar chocolate (0.13 µg/kg), followed by dark chocolate (0.084 µg/kg), milk chocolate (0.034 µg/kg), and white chocolate (< 0.04 µg/kg), which may be associated with the cocoa solids content (Fig. 2). Similar behavior was reported by Copetti et al. [29], who observed higher OTA concentrations in cocoa powder (0.92 µg/kg), followed by dark chocolate (0.87 µg/kg) and cocoa butter (0.60 µg/kg).

Table 4 OTA concentrations (mean and range) of bean-to-bar chocolates from different Brazilian regions

Origin	Positive for OTA	Mean (range) µg/kg
AM ($n = 10$)	2	0.035[a] (ND–0.19)
AP ($n = 2$)	0	ND (ND–ND)
BA ($n = 32$)	6	0.094[a] (ND–1.11)
ES ($n = 12$)	5	0.11[a] (ND–0.48)
PA ($n = 4$)	1	0.060[a] (ND–0.24)
RO ($n = 2$)	0	ND (ND–ND)
Composition	Positive for OTA	Mean (range) µg/kg
White chocolate ($n = 2$)	0	ND (ND–ND)
Milk chocolate ($n = 16$)	3	0.034[a] (ND–0.38)
Semi-sweet chocolate ($n = 13$)	3	0.13[a] (ND–1.11)
Dark chocolate ($n = 31$)	8	0.084[a] (ND–0.58)

Milk chocolate: 40–56% cocoa solids; Semi-sweet chocolate: 40–68% cocoa solids; Dark chocolate: 70–100% cocoa solids; ND = not detected. OTA < 0.04 µg/kg

[a]Different letters in samples from the same origin or composition indicate significant differences between bean-to-bar chocolates (one-way ANOVA + Tukey test, $p < 0.05$). (one-way ANOVA + Tukey test, $p < 0.05$)

The occurrence of OTA in bean-to-bar chocolates is still scarce in the literature. Concerning conventional chocolates, Copetti et al. [29] reported OTA values similar to the present study, ranging from 0.03 to 0.39 µg/kg for various products, such as cocoa powder, cocoa butter, and conventional white, milk, and dark chocolates. Chocolates made with ingredients such as oilseeds (hazelnuts, peanuts, among others) have been studied by Kabak [6] and Calderón et al. [25] in Turkey and Chile, respectively, with no positive results for OTA. In the present study, only three samples of bean-to-bar chocolates containing other ingredients were positive for OTA, including milk chocolate with hazelnuts (0.43 µg/kg), chocolate with dehydrated bananas (0.48 µg/kg), and chocolate with coconut milk (0.16 µg/kg).

The OTA concentrations of the bean-to-bar chocolate samples are in line with the maximum limits of 5 µg/kg and 3 µg/kg, respectively, established by the Brazilian [8] and European (EU, 2023) legislations for chocolates.

Exposure assessment

To determine the estimated exposure of OTA from the consumption of bean-to-bar chocolates, the weekly intake of 110.25 g for adults and children with standard body weight (60 kg and 15 kg, respectively) and the highest OTA level in the chocolate sample analyzed (1110 ng/kg—semi-sweet chocolate) were considered, which were compared to the

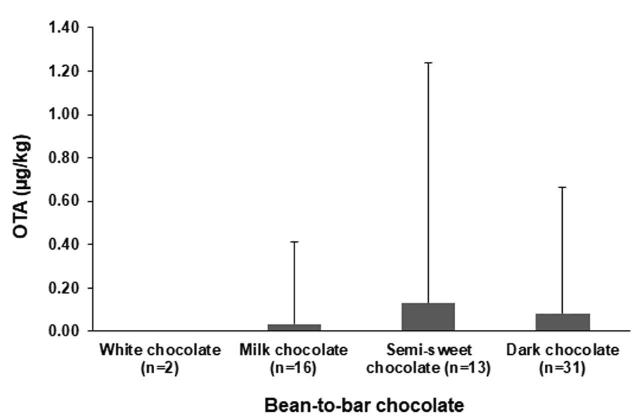

Fig. 2 Ochratoxin A levels in Bean-to-bar chocolates from Brazil

provisional tolerable weekly intake of 112 ng/kg bw [10]. The results revealed safe dietary exposure, with contributions of 1.8 and 7.3% of the PTWI for adults and children, respectively. In general, the results agree with the consensus about the dietary intake of OTA, with cocoa contributing between 5 and 6% of the total dietary exposure to OTA. (Taniwaki et al., [30]).

However, it is worth mentioning that this estimate considers a unique source of exposure (bean-to-bar chocolate), while the diet of those individuals may contain other foods with a positive occurrence of OTA, such as coffee, dried fruits, and cereals, among others. Although Bean-to-bar chocolate was considered safe for consumption, our research team has previously reported higher levels of OTA in Brazilian diet foodstuff. Black pepper samples from Brazil had levels of OTA that ranged from 0.05 to 13.15 ug/kg, according to Da Silva et al. [31]. The presence of OTA in artisanal cheese was recently reported by Marcelão et al. [32], where 22% of samples had levels between 1.0 and 1000 ug/kg. Therefore, it is important to monitor this contaminant to ensure the safety of consumers.

Conclusions

Thirty percent of cocoa beans destined for the manufacture of bean-to-bar chocolates evaluated in this study presented OTA, with the highest levels observed for samples from the AM (1.07 µg/kg) and BA (1.17 µg/kg) regions. Concerning the stages of post-harvesting processing, in which contamination with OTA has been identified as a predominant problem, only samples from the drying stage exhibited positive results (1.08 and 1.17 µg/kg). Regarding the bean-to-bar chocolates purchased from e-commerce, 23% of the samples were positive for OTA, with the highest levels observed for the chocolates produced with cocoa beans from BA (1.11 µg/kg) and ES (0.48 µg/kg). The highest occurrence of this mycotoxin was found in the samples with cocoa solids contents above 50% (semi-sweet and dark chocolate). Despite representing a growing market, little data is available in the literature on the occurrence of OTA in bean-to-bar chocolates. Therefore, monitoring this contaminant is necessary to ensure consumer safety.

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Authors' contributions V.L. Burgon: Conceptualization; Data curation; Formal analysis; Investigation; Validation; Roles/Writing—original draft. A.R.P. da Silva: Data curation; Formal analysis; Methodology. R.F. Milani: Investigation; Visualization; Writing—review & editing. M.H. Taniwaki: Conceptualization; Resources; Writing—review & editing. B.T. Iamanaka: Conceptualization; Data curation; Methodology; Resources; Supervision; Validation; Writing—review & editing. M.A. Morgano: Conceptualization; Funding acquisition; Investigation; Project administration; Resources; Supervision; Visualization; Writing—review & editing.

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Data Availability Data will be made available on request.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflicts of interest The authors declare no conflict of interest.

References

1. Giller M (2017) Bean-to-bar chocolate: America's craft chocolate revolution: the origins, the makers, and the mind-blowing flavors. Storey Publishing, North Adams
2. Agriopoulou S, Stamatelopoulou E, Varzakas T (2020) Advances in occurrence, importance, and mycotoxin control strategies: Prevention and detoxification in foods. Foods 9(2):137. https://doi.org/10.3390/foods9020137
3. Copetti MV, Pereira JL, Iamanaka BT, Pitt JI, Taniwaki MH (2010) Ochratoxigenic fungi and ochratoxin A in cocoa during farm processing. Int J Food Microbiol 143(1–2):67–70. https://doi.org/10.1016/j.ijfoodmicro.2010.07.031
4. Pires PN, Vargas EA, Gomes MB, Vieira CBM, Santos EAD, Bicalho AAC, Trovatti UAP (2019) Aflatoxins and ochratoxin A: occurrence and contamination levels in cocoa beans from Brazil. Food Addit Contam Part A 36(5):815–824. https://doi.org/10.1080/19440049.2019.1600749
5. Brera C, Debegnach F, De Santis B, Iafrate E, Pannunzi E, Berdini C, Miraglia M (2011) Ochratoxin A in cocoa and chocolate products from the Italian market: Occurrence and exposure assessment. Food Control 22(10):1663–1667. https://doi.org/10.1016/j.foodcont.2011.03.026
6. Kabak B (2019) Aflatoxins and ochratoxin A in chocolate products in Turkey. Food Addit Contam Part B Surveill 12(4):225–230. https://doi.org/10.1080/19393210.2019.1601641
7. Ostry V, Malir F, Toman J, Grosse Y (2017) Micotoxins as human carcinogens-the IARC Monograps classification. Mycotoxins Res 33:65–73. https://doi.org/10.1007/s12550-016-0265-7
8. BRASIL (2022) Ministério da Saúde. Instrução Normativa - IN n 160, de 1 de julho de 2022.
9. Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. http://data.europa.eu/eli/reg/2023/915/oj
10. JECFA (2007) Joint FAO/WHO Expert Committee on Food Additives. Sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives.
11. Fabiani A, Corzani C, Arfelli G (2010) Correlation between different clean-up methods and analytical techniques performances

to detect Ochratoxin A in wine. Talanta 83(1):281–285. https://doi.org/10.1016/j.talanta.2010.08.027

12. Quintela S, Villarán MC, Armentia IL, Elejalde E (2011) Occurrence of ochratoxin A in Rioja alavesa wines. Food Chem 126(1):302–305. https://doi.org/10.1016/j.foodchem.2010.09.096
13. Campone L, Piccinelli AL, Celano R, Pagano I, Russo M, Rastrelli L (2018) Rapid and automated on-line solid phase extraction HPLC-MS/MS with peak focusing for the determination of ochratoxin A in wine samples. Food Chem 244:128–135. https://doi.org/10.1016/j.foodchem.2017.10.023
14. Rodriguez-Cabo T, Rodriguez I, Ramil M, Cela R (2016) Liquid chromatography quadrupole time-of-flight mass spectrometry selective determination of ochratoxin A in wine. Food Chem 199:401–408. https://doi.org/10.1016/j.foodchem.2015.12.036
15. Pichon V, Combes A (2016) Selective tools for the solid-phase extraction of Ochratoxin A from various complex samples: Immunosorbents, oligosorbents, and molecularly imprinted polymers. Anal Bioanal Chem 408(25):6983–6999. https://doi.org/10.1007/s00216-016-9886-0
16. Muñoz-Solano B, González-Peñas E (2020) Mycotoxin determination in animal feed: An LC-FLD method for simultaneous quantification of aflatoxins, ochratoxins and zearelanone in this matrix. Toxins 12(6):374. https://doi.org/10.3390/toxins12060374
17. Amelin VG, Karaseva NM, Tretyakov AV (2013) Simultaneous determination of trichothecene micotoxins, ochratoxin A, and zearalenone in grain and products of its processing, feed premixes, and meat by gas chromatography. J Anal Chem 68(1):61–67. https://doi.org/10.1134/S1061934813010036
18. Xiao MW, Bai XL, Liu YM, Yang L, Liao X (2018) Simultaneous determination of trace Aflatoxin B1 and Ochratoxin A by aptamer-based microchip capillary electrophoresis in food samples. J Chromatogr A 1569:222–228. https://doi.org/10.1016/j.chroma.2018.07.051
19. Abreu DCP, Vargas EA, da Silva Oliveira FA, Madureira FD, Gomes MB, Bazzana MJF, Sack AA (2023) Validation and estimation of uncertainty of an LC-MS/MS method for the simultaneous determination of 34 mycotoxins in cocoa beans. Food Chem 399:133902. https://doi.org/10.1016/j.foodchem.2022.133902
20. Turcotte A, Scott PM, Tague B (2013) Analysis of cocoa products for ochratoxin A and aflatoxins. Mycotoxin Res 29:193–201. https://doi.org/10.1007/s12550-013-0167-x
21. MDCI (2018) Ministério da Indústria, Comércio Exterior e Serviços. Cacau e Chocolate no Brasil: Desafios na Produção e Comércio Global, 1–128. https://unesdoc.unesco.org/ark:/48223/pf0000366689/. Accessed 24 January 2023
22. INMETRO (2020) Instituto Nacional de Metrologia, Qualidade e Tecnologia. DOQ-CGCRE- 009 - Orientação sobre validação de métodos analíticos.
23. AOAC International (2016) Official methods of analysis of AOAC International, in Guidelines for Standard Method Performance Requirements (Appendix F).
24. Kulahi A, Kabak B (2020) A preliminary assessment of dietary exposure of ochratoxin A in Central Anatolia Region. Turkey Mycotoxin Res 36:327–337. https://doi.org/10.1007/s12550-020-00397-6
25. Calderón R, Palma P, Godoy M, Vidal M, Rivera A (2023) Co-occurrence and estimation of the risk of total aflatoxins (B1, B2, G1, and G2) and ochratoxin A in agri-food products consumed in Chile. Food Control 146:109493. https://doi.org/10.1016/j.foodcont.2022.109493
26. Dano SD, Manda P, Dembélé A, Abla AMJK, Bibaud JH, Gouet JZ, Sika CBZM (2013) Influence of fermentation and drying materials on the contamination of cocoa beans by ochratoxin A. Toxins 5(12):2310–2323. https://doi.org/10.3390/toxins5122310
27. Kedjebo KBD, Guehi TS, Kouakou B, Durand N, Aguilar P, Fontana A, Montet D (2016) Effect of post-harvest treatments on the occurrence of ochratoxin A in raw cocoa beans. Food Addit Contam Part A 33(1):157–166. https://doi.org/10.1080/19440049.2015.1112038
28. Delgado-Ospina J, Molina-Hernandez JB, Viteritti E, Maggio F, Fernández-Daza FF, Sciarra P, Serio A, Rossi C, Paparella A, Chaves-López C (2022) Advances in understanding the enzymatic potential and production of ochratoxin A of filamentous fungi isolated from cocoa fermented beans. Food Microbiol 104:103990. https://doi.org/10.1016/j.fm.2022.103990
29. Copetti MV, Iamanaka BT, Pereira JL, Lemes DP, Nakano F, Taniwaki MH (2012) Co-occurrence of ochratoxin A and aflatoxins in chocolate marketed in Brazil. Food Control 26(1):36–41. https://doi.org/10.1016/j.foodcont.2011.12.023
30 Taniwaki MH, Pitt JI, Copetti MV, Teixeira AA, Iamanka BT (2019) Understanding mycotoxin contamination across the food chain in Brazil: Challenges and Opportunities. Toxins. 11:411. https://doi.org/10.3390/toxins11070411
31 Da Silva ARP, Fungaro MHP, Silva JJ, Martins LM, Taniwaki MH, Iamanaka BT (2021) Ochratoxin A and related fungi in Brazilian black pepper (*Piper nigrum L.*). Food Res Int. 142:110207. https://doi.org/10.1016/j.foodres.2021.110207
32. Marcelão CVP, Souza MC, Silva JJ, Couto FA, Lacorte GA, Pinto UM, Maffei JT, Zacarchenco PB, Iamanka BT, Taniwaki MH (2024) Unveiling ochratoxin A and ochratoxigenic fungi in Brazilian artisanal Cheeses: Insights from production to consumption. Food Res Int. 183:114214. https://doi.org/10.1016/j.foodres.2024.114214