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ORGINAL ARTICLE

Coffee and wine with ochratoxin A – exposure risk assessment resulting from its consumption

Zanieczyszczenie kawy i wina ochratoksyną A – ocena narażenia wynikająca z jej spożycia

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A – Koncepcja i projekt badania, B – Gromadzenie i/lub zestawianie danych, C – Analiza i interpretacja danych, D – Napisanie artykułu, E – Krytyczne zrecenzowanie artykułu, F – Zatwierdzenie ostatecznej wersji artykułu

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Abstract

Objective. The aim of this study was to assess the consumer risk resulting from the exposure to ochratoxin A (OTA) intake with coffee and wine.

Materials and method. Calculations were made on the basis of the levels of OTA contamination determined in coffee and wine available in retail in the Małopolskie province of Poland in 2016–2019. A total of 56 samples were analysed: 24 wine samples (20 red wines and 4 white wines), 16 samples of roasted coffee beans or ground coffee, and 16 samples of instant coffee. All samples were examined for the level of OTA contamination by high-performance liquid chromatography with fluorescence detection (HPLC-FLD).

Results. Of the 56 samples examined, ochratoxin A was found in 16 samples (28.6%). Among the tested samples, this toxin was most often detected in instant coffee, i.e. in 10 of 16 samples (62.5%), average level – 1.63 μg kg^{-1}. Average level of OTA in roasted coffee – 1.09 μg kg^{-1}, and presence of OTA confirmed in 5 out of 16 samples (31.3%). In contrast, in wine, only one of the 16 samples had this toxin (0.61 μg l^{-1}).

Conclusions. OTA contamination levels observed in all examined products were below the maximal residue levels (MRL) specified in the regulations of food law for these products. Assessment of consumer exposure to OTA resulting from the intake of this toxin from coffee and wine, in general showed that consumption of these products is safe; however, under extreme assumptions of high consumption of these products, the permissible OTA intakes will be exceeded.

Key words

ochratoxin A, health risk assessment, mycotoxins, wine, coffe, beverages

Streszczenie

Wprowadzenie i cel pracy. Celem niniejszej pracy była ocena narażenia wynikająca ze spożycia ochratoksyny A (OTA), zawartej w kawie oraz winie.

Materiał i metody. Obliczenia wykonano na podstawie stwierdzonych poziomów zanieczyszczenia ochratoksyną A kawy oraz wina dostępnych w handlu detalicznym na terenie województwa małopolskiego w latach 2016–2019. Łącznie przebadano 56 próbek, a mianowicie: 24 próbki wina (20 win czerwonych oraz 4 białe), 16 próbek palonych ziaren kawy lub kawy mielonej oraz 16 próbek kawy rozpuszczalnej. Poziom zanieczyszczenia ochratoksyną A w badanych próbkach określono, posługując się metodą wysokosprawnej chromatografii cieczowej z detekcją fluorescencyjną (HPLC-FLD).

Wyniki. Wśród 56 badanych próbek ochratoksynę A wykryto w 16, co stanowiło 28,6%. Spośród badanych próbek najczęściej toksynę tę stwierdzano w próbkach kawy rozpuszczalnej, tj. znajdowała się ona w 10 z 16 próbek (62,5%), zaś średni jej poziom wynosił 1,62 μg/kg. W próbkach kawy palonej średni poziom ochratoksyny A wyniósł 1,08 μg/kg, przy czym jej obecność stwierdzono w 5 z 16 próbek (31,3%). Natomiast w winie tylko w jednej z 16 próbek stwierdzono tę toksynę, przy czym jej poziom wynosił 0,6 μg/l.

Wnioski. Zaobserwowane poziomy zanieczyszczenia we wszystkich badanych asortymentach były niższe od najwyższego dopuszczalnego poziomu (NDP) określonego w przepisach prawa żywnościowego dla tych produktów. Ocena narażenia konsumentów na OTA, przeprowadzona na podstawie pobrania tej toksyny z kawy oraz wina, wskazała generalnie na bezpieczeństwo spożycia tych produktów, jednak przy skrajnych założeniach dużej ich konsumpcji przekroczono dopuszczalne poziomy pobrania.

Słowa kluczowe

ochratoksyna A, ocena ryzyka, mykotoksyny, wino, kawa, napoje

INTRODUCTION

Ochratoxin A (OTA) is one of the most common mycotoxins occurring in food [1]. It is produced by various fungal species

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belonging to the genera *Penicillium* and *Aspergillus* [2]. OTA is present in many food products, such as spices, coffee, cereals, beans and fruits, and in the products obtained from them [3]. Ochratoxin A has been classified by the International Agency for Research on Cancer (IARC) in group 2B, as a possible human carcinogen. This resulted in the rise of concern about human risk exposure to this mycotoxin [4]. In addition, this toxin exhibits neurotoxic, nephrotoxic and immunosuppressive effects [5,6].

Due to the high consumption of coffee worldwide, its contribution in the overall OTA intake is about 12%, and is therefore considered to be the third source of the OTA exposure. The main source of the OTA exposure are cereals and cereal products, while wine occupies second place [7]. In 2018, global wine production amounted to 292.3 million hl, while global consumption was estimated at 246 ml hl [8]. Over the past ten years, wine consumption in Poland has been between 6.0–6.9 litres *per capita* [9]. OTA occurrence in wine is mainly due to grape contamination by *Aspergillus carbonarius* and *Aspergillus niger* while still in the vineyard, or at stages preceding wine production. The OTA level in the final product is also affected by the following factors: grape variety, its damage, location of the vineyard, temperature, relative humidity, rainfall, microclimate, the harvest period, use of plant protection products, good agricultural practice in the vineyard, oenological stages, and good wine production practices [5]. Ongoing studies have shown that with regard to wine, the geographical region of origin affects the frequency of prevalence and content of OTA. For example, in Europe, the amount of OTA in wine obtained from southern regions is higher than that from northern areas due to the warmer climatic conditions [10,35].

Coffee is a hot beverage prepared by brewing properly processed coffee beans of the coffee tree (mainly Coffea canephora or robusta and Coffea arabica). Globally, coffee is second in the ranking of consumed hot beverages. This position can be explained by the unique mix of sensory and physiological properties resulting from the wide variety of its chemical constituents [11,12]. Coffee drinks are very popular worldwide and coffee is the second largest food commodity traded. In 2019, the European Union (EU) imported almost three million tonnes of coffee [13]. Global coffee production in 2019–2020 is estimated at 169.34 million bags, while its global consumption is around 167.81 million bags [14].

Fungal contamination of coffee beans can occur at any stage of the production chain (before harvest, during storage, transport). If coffee beans are contaminated with OTA, the toxin is not completely destroyed during the roasting process; thus, the parameters of the coffee roasting process have also an effect on the level of OTA contamination in the end product [15,16]. In European legislation, maximum levels of ochratoxin A are regulated, for example, by Commission Regulation (EC) No. 1881/2006 of 19 December 2006, setting maximum levels for certain contaminants in foodstuffs. According to this regulation, the OTA contamination is set at 5.0 μg kg^{-1} for roasted coffee beans and ground coffee, at 10.0 μg kg^{-1} for soluble coffee, and at 2.0 μg kg^{-1} for wine [17]. In May 2020, the European Food Safety Authority (EFSA) published a scientific opinion on the public health risks resulting from the presence of OTA in food. In this opinion, a more conservative approach was used to calculate the margin of OTA exposure (MOE), which led to the conclusion that its presence in food is responsible for health problems in the majority of the consumer groups. The scientific opinion of EFSA was forwarded to the European Commission, and discussions are currently ongoing on maximum allowable levels of OTA in foodstuffs [18]. Currently, there is also no literature data on the presence of ochratoxin A in coffee and wine available on the retail market in Poland. Therefore, the aim of this study was to assess consumer risk resulting from the exposure to ochratoxin A intake with coffee and wine, available in retail in the Lesser Poland Region in 2016–2019.

Experimental material. The experimental material consisted of roasted coffee beans, ground roasted coffee, instant coffee and wine, obtained from retail sales in the Lesser Poland Province in 2016–2019. A total of 56 samples were analysed, including 24 wine samples (20 red wines and 4 white wines), 16 samples of roasted coffee beans or ground coffee, and 16 samples of instant coffee. All samples, collected by inspectors of sanitary-epidemiological stations in the Lesser Poland Province according to the rules set in Commission Regulation (EC) 401/2006 [19], were examined for the level of OTA contamination by high-performance liquid chromatography with fluorescence detection (HPLC-FLD).

Sample preparation procedure.

Coffee. A 5 g homogeneous sample was weighed and mixed with 100 ml of 1% aqueous sodium bicarbonate solution for about 2 min at high speed. The sample was then filtered through a filter paper and the filtrate collected in a conical flask. 20 ml of the mixed filtrate was diluted with 20 ml of phosphate buffer in saline (PBS). The sample extract thus diluted was then passed through an Ochraprep® immunoaffinity column (IAC) at the a rate of approx. 1 ml min^{-1}. Afterwards, the column was washed with 20 ml of PBS at approx. 5 ml min^{-1} and air dried for about 15 sec under reduced pressure. Ochratoxin A was eluted into a chromatographic vial by passing 1.5 ml of desorbating solution (methanol/acetic acid, 98:2, v/v) through the column at the rate of approx. 1 drop sec^{-1}. The eluate (3.0 ml) was collected prior to analysis by HPLC.

Wine. 10 ml of wine was adjusted to pH 8.2 with 0.1 M sodium hydroxide and diluted with 10 ml of PBS buffer. The extract thus diluted was passed through an Ochraprep® immunoaffinity column at the rate of approx. 1 ml min^{-1}. The column was then washed with 20 ml of 0.01% Tween 20 (polysorbate) in PBS at the rate of approx. 5 ml min^{-1} and air dried under reduced pressure for approx. 15 s. Ochratoxin A was eluted into the chromatographic vial with 1.5 ml of desorbing solution (methanol/acetic acid, 98: 2, v/v) at the rate of approx. 1 drop / sec^{-1}. The collected eluate (3.0 ml) was subjected to chromatographic analysis by the HPLC-FLD method.

Chemicals. Methanol, sodium bicarbonate, acetic acid, acetonitrile, sodium hydroxide, Tween 20 (polysorbate), and phosphate buffer were purchased from Sigma-Aldrich (Poznań, Poland)**.** All reagents were of pro-analysis grade. Ochraprep® immunoaffinity columns (IAC) were obtained from 'FABIMEX' Więcek Sp. j. In addition, 1% sodium bicarbonate solution was prepared by dissolving 10 g of sodium bicarbonate in 1 l of redistilled water. Phosphate buffer in saline (PBS) was prepared by dissolving 8.0 g sodium chloride, 1.2 g sodium hydrogen phosphate, 0.2 g potassium

and 0.2 g potassium chloride in 990 ml of redistilled water. The pH was then adjusted to 7.4 with 0.1 M sodium hydroxide solution and the whole was made up to 1,000 ml with redistilled water.

Apparatus and chromatographic conditions. Determination was conducted in accordance with own research procedure developed on the basis of: PN-EN 14132: 2010 and PN-EN 14133: 2010 [20,21]. Ochratoxin A quantification was carried out using a SHIMADZU liquid chromatograph equipped with a FLD fluorescence detector. The analytical conditions were: the mobile phase consisting of acetic acid (9% solution) and methanol (28:72, v/v); column operating temperature: 35°C; flow rate: 1 ml min^{-1}; injection volume: 20 µL, excitation wavelength – λ = 333 nm, and emission wavelength – λ = 460 nm.

Dietary exposure assessment. Based on the results obtained, consumers risk exposure through consumption of the examined products was estimated with reference to the EFSA values of the average chronic OTA exposure, which were set for adults within the range 2.41 LB (lower bound) and 5.09 ng kg^{-1} bw day^{-1} UB (upper bound). The obtained values were expressed as a % of these values (Tab. 3). The exposure dose was calculated according to the following formula:

(µg kg^{-1} body weight per day) = mycotoxin level (µg kg^{-1}) × average food intake (kg per person per day) × average body $weight^{-1}$ (kg^{-1} body weight per person).

With regard to roasted coffee, the results obtained have been calculated on the basis of the middle bound (MB) value (0.14 µg kg^{-1}) and the highest value (1.53 µg kg^{-1}) of OTA contamination. The MB value was calculated as the difference between upper bound ($UB^{*)}$) and lower bound (LB^{*}). LB^{*} and UB^{*} values are applied when the percentage of results below the Limit of Detection (LOD) or Limit of Quantitation (LOQ) is higher than 50%. According to the guidelines of the European Food Safety Authority [22], at the (LB^{*}, results below the LOD were replaced by zero and those below the LOQ by the LOD; at the upper-bound (UB^{*}) the results below the LOD were replaced by the value of the L, and those below the LOQ were replaced by the value reported as LOQ.

In calculations, based on the studies of Santini et al. [23], an assumption was made that 80% of the OTA contained in coffee passes into prepared drink and the brew is usually prepared with 2 teaspoons (10 g) of ground coffee [24]. For ground coffee calculations, the above was one portion; for instant coffee, the assumption was that 12 g of coffee (two 6-gram teaspoons) is used to prepare this drink. In this case, calculations were also based on the mean value and the highest contamination value, which this time were 1.63 µg kg^{-1} and 2.77 µg kg^{-1}, respectively.

In the case of wine, the obtained level of highest contamination (0.61 µg l^{-1}) and MB value (0.147 µg kg^{-1}) was assumed. The calculations performed also concerned the total consumption pattern of these products, i.e., 3 coffees (1 roasted and 2 instant) a day, and one glass of wine. Calculations were based on the values of average body weight taken from the Nutritional Standards for the Polish population, which were developed for 2 groups of adults: men (A) and women (B), aged 19 and over. The respective values for these groups were 70.0 kg and 60.0 kg [25].

RESULTS AND DISCUSSION

Among the 56 tested samples, ochratoxin A was determined in 16 samples (28.6%) (Tab. 1). In these samples, there was 10 (62.5%) were of instant coffee in which the toxin was found most often at the average level of 1.63 ± 0.84 µg kg^{-1}, as well as 5 samples (31.3%) of roasted coffee, in which the mean OTA level was 1.09 ± 0.33 µg kg^{-1}. In contrast, in wine, this toxin was found in only 1 of 16 samples at the level of 0.61 ± 0.02 µg l^{-1}. In all examined samples, the contamination levels were below the maximum permissible level specified in Regulation No. 1881/2006 [17]. The findings of the current study are in agreement with those of Pierzynowska et al. [24], who reported similar levels of OTA contamination (1.51 ± 0.09 µg kg^{-1}) for instant coffee available in Poland, while the content of this toxin in ground coffee was higher – 1.49 ± 0.10 µg kg^{-1}. The contents of ochratoxin A in coffee from Argentina noted by Drunday and Pacin [26], were close to the aforementioned. According to the authors, who examined 51 coffee samples (green coffee, roasted coffee beans and instant coffee samples), the average contamination levels of roasted and instant coffee were 1.00 ± 1.54 and 1.99 µg kg^{-1} respectively. The authors found that 69% of the coffee samples were contaminated with OTA at different levels, but only 3 did not meet the requirements of European Union regulations [17] (Tab. 2A). In turn, studies of Vecchio et al. [27] showed OTA presence in 96% (48 samples) of examined Italian instant coffee samples at the range of 0.32–6.40 µg kg^{-1}; mean value – 1.27 µg kg^{-1}. Nevertheless, all examined samples met the requirements set out in EU regulations regarding the maximum permissible level of OTA contamination.

When analysing the levels of coffee contamination with ochratoxin A in global perspective, higher contamination levels were stated by Casal et al. [28], who examined instant coffee and its substitutes (different types of cereal coffees) available in Portugal. The authors examined 10 samples of these products, and ochratoxin A content was at the average level of 2.50 µg kg^{-1} in 90% of the types of coffee. The values ranged from 0.16–11.8 µg kg^{-1}, with the acceptable level exceeded in only one case. Additionally, Coronel et al.

Table 1. Level of ochratoxin A contamination of the examined samples

Product	No. of positive samples/ examined samples (%)	Range (min-max, µg kg^{-1})	Mean of positive samples (µg kg^{-1})	Mean MB (UB-LB, µg kg^{-1})	UE ML (µg kg^{-1})	No. of samples above MRL
Roasted coffee beans and ground roasted coffee	5/16 (31.3)	0.61–1.53	1.09	0.14 (0.48–0.34)	5.0	0
Instant coffee	10/16 (62.5)	0.61–2.77	1.63	nc	10.0	0
Wine	1/24 (4.2)	0.61	0.61	0.147 (0.172–0.025)	2.0	0

nc – not calculated

Table 2A. Concentration of ochratoxin A in various types of coffee.

Food sample	Contamination level [μg kg^{-1}]	Average contamination level ± SD [μg kg^{-1}]	Year	Country	Percentages of samples with contamination level above UE limit	References
Roasted coffee	0.85–2.50	1.49 ± 0.10	2016	Poland	0	Pierzynowska, et al., 2016
Instant coffee	0.84–2.07	1.51 ± 0.09			0	
Roasted coffee	0.11–5.78	1.00 ± 1.54	2013	Argentina	nc	Drunday and Pacin, 2013
Instant coffee	0.22–13.66	1.99 ± 3.74				
Roasted coffee	0.30–0.84	0.47 ± 0.20	2014	Chile	0	Galarce-Bustos, et al., 2014
Instant coffee	0.28–5.58	1.80 ± 1.81			0	
Instant coffee	0.16–11.8	2.20 ± 3.53	2014	Portugal	10	Casal, et al., 2014
Roasted coffee	1.21–4.21	2.17 ± 0.79	2011	Spain	0	Coronel et al., 2011
Roasted coffee	0.71–10.31	1.84 ± 0.03	2017	Portugal	16.7	Benites et al., 2017
Instant coffee	7.16	1.45 ± 0.02			20.0	
Instant coffee	0.32–6.40	1.27 ± 1.03	2012	Italy	0	Vecchio et al., 2012
Roasted coffee	nc	2.3	2015	Denmark	8.8	Nielsen et al., 2015
Instant coffee	nc	4.5		Kenya. Tanzania. Uganda	0	

nc – not calculated, SD – standard deviation

[29], in studies on the roasted coffee available in Spain, also proved the OTA presence in 35 out of 72 examined samples (48.6%); the amount ranged from 1.21–4.21 μg kg^{-1}, mean value – 2.17 μg kg^{-1}. In turn, Benites et al. [30] determined OTA contamination in roasted coffee beans and ground coffee available in retail in Portugal. Out of the 6 samples of roasted coffee beans, the toxin was found in 2, while in ground coffee samples in 1 out of 5. The mean concentrations in roasted beans and ground coffee samples were 1.84 μg kg^{-1} and 1.45 μg kg^{-1}, respectively. Nielsen et al. [31], who analysed green, roasted and soluble coffee samples, detected this toxin at the average level of 2.3 μg kg^{-1}, in 46% of roasted coffee samples from the Danish retail market. Only 5 samples exceeded the limit set in the EU, and 1 sample showed the level to be as high as 21 μg kg^{-1}. On the other hand, none of the 25 instant coffee samples from Kenya, Tanzania and Uganda had a higher OTA level than the limit set by European legislation (10 μg kg^{-1}), and its presence at an average level of 4.5 μg kg^{-1}was found in 56% of the tested samples.

The lower mean OTA level (0.47 μg kg^{-1}) was determined by Galarce-Bustos [7] in the 39 samples of instant coffee commercially available in Chile. With regard to roasted coffee, the mean value (1.80 μg kg^{-1}) obtained for 24 samples was higher. Due to the lack of legal regulations in force in Chile regarding the OTA contamination of coffee, the results obtained were compared to the European requirements. In no case was the limit applicable for these products on the European market exeeded.

As for wine contamination with ochratoxin A, OTA levels determined in wine in this study were higher than the levels reported by Čepo et al. [2] for Croatian wines (Tab. 2B). The authors confirmed the OTA presence in the range between 0.01–0.24 μg l^{-1} in 30.8% of the examined samples (i.e. 8 out of 26 samples). Lower OTA levels were also determined by Hajok et al. [32] in wines available in retail in Poland, with the toxin being found in 25% of the examined wine samples (i.e. 8 of 32 samples), at the average level of 0.1 μg $^{l-1}$. Lower OTA levels were also found in studies of Quinteli et al. [10], who detected this toxin in 88% of wines imported from the UK and Germany, in 100% of wines from Switzerland and 67% of wines originating from the USA. The average OTA concentrations were as follows: 0.051 μg l^{-1}, in wines from the UK; 0.085 μg l^{-1}, in those from Germany; 0.125 μg l^{-1}, in wines from the USA; and 0.149 μg l^{-1}, in wines from Switzerland. In none of the examined samples the OTA content exceeded the maximum level set in the EU. In turn, Vega et al. [33], who investigated wines commercially available in Chile, showed OTA presence in only 2.9% of all samples. The highest concentration was 0.15 ± 0.11 μg l^{-1} and concerned only a single sample. A similar level of ochratoxin A was noted by Oteiza et al. [34] who examined OTA contamination of wines available in Argentina. The toxin was detected in 0.2% of the examined samples (i.e., 2 out of 801 samples), with the highest concentration at 0.5 μg l^{-1}. Remiro et al. [35], who investigated the co-occurrence of Ochratoxin A (OTA) and its 5 analogues (OTB, OTC, MeOTA, MeOTB and EtOTB) samples of Mediterranean red wine, showed that ochratoxin A was present in 99% of the samples analysed (i.e. 95 samples). None of the samples had an OTA level higher than the maximum limit set by EU legislation. The highest value detected was 0.455 μg l^{-1} and concerned to wine from North Africa. The mean value obtained was 0.054 μg l^{-1}. Taking into account the OTA content and country of origin, the wines examined were ordered (in ascending order) as follows: Israel < Croatia < Turkey < France < Spain < Italy < Greece < North Africa. For every wine, 2 values are given: a mean OTA concentration and the range of the results obtained. The lowest OTA content (0.0185 μg l^{-1}) was observed in Israeli wines, in which the results obtained ranged from 0.0036–0.0654 μg l^{-1}, followed by Croatian red wines: 0.0188 μg l^{-1}, 0.0004–0.0613 μg l^{-1}; Turkish wines: 0.0311 μg l^{-1}, 0.0029–0.101 μg l^{-1}; French wines: 0.0336 μg l^{-1}, 0.0002–0.0883 μg l^{-1}; Spanish wines: 0.0372 μg l^{-1}, 0.001–0.104 μg l^{-1}; Italian wines: 0.0539 μg l^{-1}, 0.0052–0.286 μg l^{-1}; Greek wines: 0.0594 μg l^{-1}, 0.0004–0.212 μg l^{-1}); and finally North Africa wines with a mean OTA concentration of 0.195 μg l^{-1}. The results ranged from 0.0844–0.455 μg l^{-1}. Thus, in all examined wines, the OTA level was lower than that recorded in the current study. A lower OTA level was also noted by Sarigiannis et al. [36] in 86.7% (i.e. 52 out of 60 samples) of wines available on the Greek market. The average concentration of this toxin was 0.38 μg l^{-1}, and in 5 samples OTA levels exceeded the limit set by EU legislation. The maximum OTA concentration found in these studies was 2.52 μg l^{-1}.

Table 2B. Concentration of ochratoxin A in wines.

Food sample	Contamination level [µg/kg[	Average contamination level ± SD [µg/kg]	Year	Country	Percentage of samples with contamination level above UE limit	References
Wine	0.010–0.237	0.051	2012	United Kingdom	0	Quintela et al., 2012
	0.060–0.138	0.085		Germany	0	
	0.030–0.353	0.125		United States	0	
	0.063–0.309	0.149		Switzerland	0	
Wine	0.05–0.35	0.15 ± 0.11	2012	Chile	0	Vega et al., 2012
Wine	0.01–0.24	Not calculated	2018	Croatia	0	Čepo et al., 2018
Wine	0.1	0.1	2019	Poland	0	Hajok et al., 2019
Wine	Not calculated	0.001± 0.02	2017	Argentina	0	Oteiza et al., 2017
Wine	0.0036–0.0654	0.0185	2013	Israel	0	Remiro et al., 2013
	0.0004–0.0613	0.0188		Croatia	0	
	0.0029–0.101	0.0311		Turkey	0	
	0.0002–0.0883	0.0336		France	0	
	0.001–0.104	0.0372		Spain	0	
	0.0052–0.286	0.0539		Italy	0	
	0.0004–0.212	0.0594		Greece	0	
	0.0844–0.455	0.195		North Africa	0	
Wine	0.01–2.52	0.38	2014	Greece	9,6	Sarigiannis et al., 2014

In all analysed cases, the daily OTA intake through the consumption of brewed coffee did not exceed the acceptable limits, and amounted to a maximum of 31.7% of this value. The limit for instant coffee was also not exceeded, although the maximum determined value was already 69%. On the other hand, ochratoxin A intake with wine, drunk in the amount of 300 ml by both men and women, was 108.5 and 126.6%, respectively. In addition, the daily OTA intake of both of coffee and wine was also analysed. For the maximum determined level of contamination, the result obtained amounted to 82.9%, and 96.7% of the established lower limit.

Torović et al. [37] showed that the exposure to OTA through wine consumption carried a very low risk to public health. In turn, Hajok et al. [32] reported that there was also no risk related to the consumption of wine and OTA-contaminated soluble coffee. According to Ostry et al. [38], the exposure to OTA through coffee and wine consumption was highest in the adult female population, but still made only a minor contribution to total OTA consumption (0.059 and 0.024 ng kg^{-1} b.w. day^{-1}). In addition, Silva et al. [39] found that OTA intake through wine consumption does not pose a risk to consumers.

The values obtained for daily OTA intake in most cases were within the established range; however, it should be remembered that this toxin is also taken up with cereal products, which are a fundamental part of the diet. Therefore, it is justified to monitor continuously the content of ochratoxin A in products.

Table 3. Daily intake of ochratoxin A

Food sample	The amount consumed	Average value of contamination or MB value				Maximum value of contamination			
		% LB in relation to the average body weight [%]		% UB in relation to the average body weight [%]		% LB in relation to the average body weight [%]		% UB in relation to the average body weight %	
		A	B	A	B	A	B	A	B
Brewed coffee	One cup	0.7	0.8	0.3	0,4	7.3	8.5	3.4	4.0
	2 cups	1.3	1.5	0.6	0.7	18.1	21.2	6.9	8.0
	3 cups	2.0	2.3	0.9	1.1	27.2	31.7	10.3	12.0
Instant coffee	One cup	11.6	13.5	5.5	6.4	19.7	23.0	9.3	10.9
	2 cups	23.2	27.1	11.0	12.8	39.4	46.0	18.7	21.8
	3 cups	34.8	40.6	16.5	19.2	59.1	69.0	28.0	32.7
Wine	1 glass (100 ml)	8.7	10.2	4.1	4.8	36.2	42.2	17.1	20.0
	2 glasses (200 ml)	17.4	20.3	8.3	9.6	72.3	84.4	34.2	39.9
	3 glasses (300 ml)	26.1	30.5	10.3	12.0	108.5	126.6	42.8	49.9
Coffee + wine	3 cups +1 glass	32.6	38.0	15.4	18.0	82.9	96.7	39.2	45.8

A – men, B – women, LB – lower bound, UB -upper bound – EFSA reference values of the average chronic OTA exposure, MB middle bound value was calculated as the difference between UB^* and LB^*. LB^* – results below the LOD were replaced by zero and those below the LOQ by the LOD; UB^* the results below the LOD were replaced by the value of the LOD and those below the LOQ were replaced by the value reported as LOQ.

CONCLUSIONS

1. Of the 56 examined samples, ochratoxin A was found in 16 samples, representing 28.6%.
2. The toxin was determined in 62.5% samples of instant coffee (i.e. in 10 out of 16 samples) at the average level of 1.63 μg kg^{-1}. In roasted coffee samples, the mean OTA level was 1.09 μg kg^{-1} and OTA presence was stated in 31.3% of samples (i.e.in 5 out of 16 samples). In wine samples, this toxin was detected at a level of 0.61 μg l^{-1}, in only 1 of the 16 samples examined.
3. The levels of contamination obtained in this study in all examined products were lower than the maximum residue level (MRL) regulated by European Union legislation.
4. The assessment of consumer exposure to OTA resulting from the intake of this toxin from coffee and wine, generally shows that consumption of these products is safe; however, under extreme assumptions of high consumption of these products, the permissible OTA intakes have been exceeded.
5. In view of the fact that ochratoxin A is also taken from cereal products, which constitute a fundamental part of the diet, it is justified to monitor continuously OTA content in these products.

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