Ochratoxin A and coffee

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Ochratoxin A and Coffee*

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Introduction

The mycotoxin ochratoxin A (OA) derives its name from *Aspergillus ochraceus*, the mould from which it first was isolated (1). It is the main toxic component in cultures of this mould, yet it is also produced by other ubiquitously found moulds such as in various other strains of Aspergillus and Penicillium. The fact that OA is produced by a variety of moulds, many of which are also found on corn, maize and cereal crop, as well as the carcinogenicity of OA found in rats (2) resulted in an increased request for a better database on the presence of OA in foodstuffs and a demand to characterise the potential health risk associated with the daily exposure of humans to OA. One of the foodstuffs that had gained attention with regard to contamination with OA is coffee. OA has been detected in green and roasted coffee beans (3–7). Yet inconsistent results have been published with respect to the influence of the roasting process on the OA content and the transfer of OA into the coffee brew (3, 5, 7, 8). It is the intention of this paper not only to present new data regarding the contamination of green coffee beans with OA and the effects of the roasting process on the OA content in roasted coffee beans and the corresponding coffee brew, but also to attempt a preliminary risk assessment of OA in the light of every day coffee consumption.

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Short Literature Review

The data published on OA in green coffee beans are shown in table 1. First analyses on this field were undertaken in 1974 but mostly with mouldy lots. The detection limits were around 20 µg OA/kg coffee. A higher percentage of contaminated samples (18–60%) also in commercial (not spoiled) beans were found when the methods of analysis were improved, respectively lower detection limits were achieved.

Table 1. Published data on the occurrence of ochratoxin A in green coffee beans

Number of Samples	Type	Samples contaminated with OA			Reference
		Nr.	(%)	Content in µg/kg	
267	hand cleaned from mouldy lots	23 5 1	8.6% 1.9% 0.4%	< 20 30–50 360	(4)
68	commercial	2 1	2.9% 1.5%	20 80	(4)
502	2 years old, one sample mouldy	0	0.0%		Illycaffe (via Levi)
201	commercial	1 1	0.5% 0.5%	24 96	FDA USA (via Levi)
40	commercial	9	22.5%	0.5–23.5	(3)
22	commercial	4	18.2%	9.9–46.0	(6)
28	commercial	17	60.7%	0.2–15.0	(5)

The data regarding the destruction of OA (see table 2) during roasting are contradictory. In four of five studies the destruction was between 50 and 100%. Only in the study of *Tsubouchi* et al. (7) the destruction of OA was around 10%. Investigations on the carry over of OA from ground roasted coffee into the coffee brew are only reported in two studies (5, 7). Again contradictory results were found. *Micco* et al. (5) could not find any OA in the coffee brew prepared from artificially contaminated coffee beans. *Tsoubuchi* et al. (7) in contrast found essentially no loss of OA during preparation of coffee brews using coffee beans contaminated by inoculation.

OA in commercially roasted beans was found in Japan (9) in 5 of 68 samples (7% positive), containing OA in the range of 3.2–17 µg OA/kg coffee (detection limit: 2 µg OA/kg coffee).

Table 2. Published data on the destruction of ochratoxin A during roasting of coffee beans

Method of contamination	Number of samples	OA content (µg/kg)	Roasting conditions	Destruction	Reference
mycotoxin added	4	210–350	198–210 °C 5–20 min	80–90%	(4)
A. ochraceus inoculation	2	17, 43	not specified	80–90%	(8)
mycotoxin added	3	80	not specified	90%	(8)
naturally contaminated	2	3.8, 23	not specified	90%	(3)
naturally contaminated	2	4.0, 8.6	not specified	90–100%	(5)
mycotoxin added	3	45	not specified	50–87%	(5)
A. ochraceus inoculation	4	200–140 000	200 °C 10–20 min	0–12%	(7)

Material and Methods

Ochratoxin A was extracted with methanol/sodium bicarbonate, purified with a celite column, detected and quantified by HPLC equipped with a fluorescence detector. Confirmations of the OA identity were carried out either via methylation of the OA molecule and subsequent HPLC analysis or by GC-MS for green coffee bean samples and by immunoaffinity column purification/HPLC or immunoaffinity column purification/GC-MS for roasted coffee beans and the coffee brew. Material and methods are described and discussed in detail by *Studer-Rohr* et al. (10).

Results and Discussion

Ochratoxin A in green commercial coffee samples

Samples of different origins, supplied by various coffee industries, were analysed. Of 25 analysed samples 13 were positive for OA and contained between 1.2 and 56 µg OA/kg coffee (detection limit: 0.5 µg OA/kg coffee), however, the sample containing 56 µg OA/kg had a rather spoiled appearance. These results are in line with the literature (table 1).

Destruction of ochratoxin A in spoiled coffee beans during roasting

Three naturally contaminated green coffee bean samples with high amounts of OA (400–1500 µg OA/kg coffee) and two samples inoculated with a spore suspension of *A. ochraceus* (90–140 µg OA/kg coffee) were divided into two batches. One batch was analysed directly for OA contamination, the other batch was roasted before analysing. Out of each batch several aliquots were analysed. The results are shown in figure 1. Substantially no statistically significant loss of OA due to roasting could be observed. However, due to the inhomogeneity of OA content within the samples (shown by the standard errors of the mean) a loss of OA during roasting between maximally 14% and 62% for the three naturally contaminated samples would not have been detected. In the two inoculated samples a much more homogenous OA distribution within the samples was achieved (as indicated by the low standard errors of the mean). In one sample no loss of OA after roasting could be seen. In the other sample a statistically significant reduction between 2% and 29% of the OA content during roasting is calculated. It is likely therefore that a reduction of the OA content during roasting of less than 30% takes place. The inconsistent results in the literature concerning the loss of OA during roasting (table 2) can be explained partially by the different methods of contamination and partially by the inhomogeneity of OA distribution within the coffee samples. If OA is only superficially added to the coffee beans it is likely that the main part of OA is removed with the loss of the silver skin of the coffee beans during roasting. This problem can be eliminated by using naturally contaminated or inoculated beans. However, especially with naturally contaminated coffee beans it should be considered that the inhomogeneity of the OA distribution within the samples is enormous (10). Therefore it is very difficult to quantify the amount of destruction when only a small numbers and low contaminated samples are used for analyses.

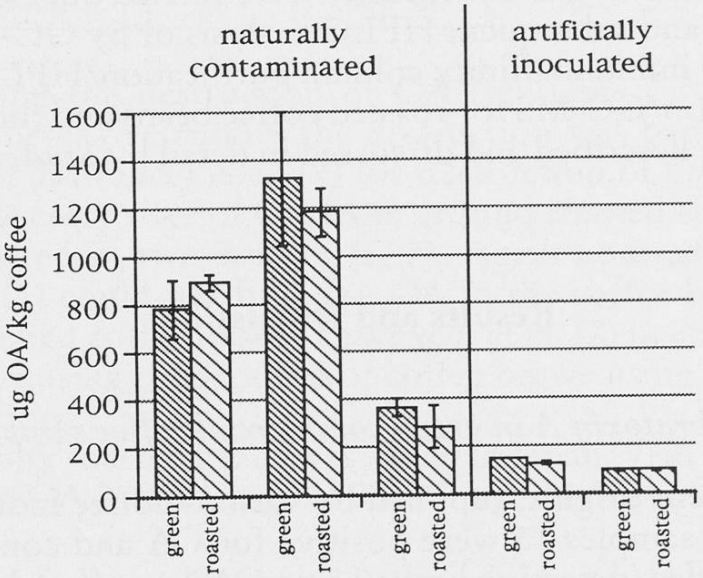

Fig. 1. OA content in naturally spoiled coffee and artificially inoculated coffee beans before (green) and after (roasted) roasting (the OA amounts are calculated based on green bean weight) (arithmetic means of 3–6 samples; ± standard error of the mean)

The heat stability of OA was further investigated in an experiment where pure OA was kept between 0 and 12 minutes at 270 °C. (Normal roasting conditions for 500 g coffee are between 250 °C and 270 °C for 120–150 seconds.) The results are shown in figure 2. Up to 6 minutes no loss of OA was detected. And even after 12 minutes 50% of OA could still be detected. These results confirm the findings of the roasting experiments.

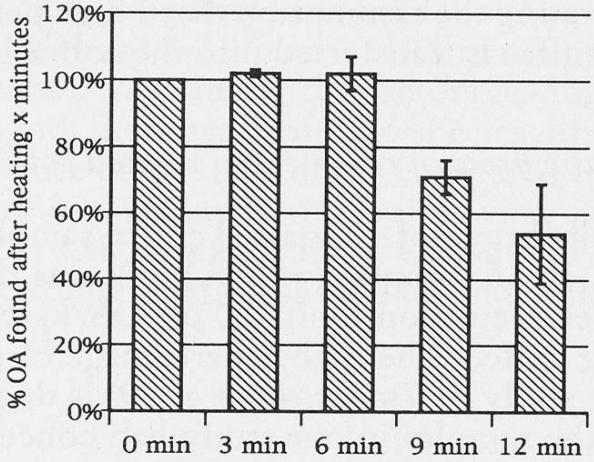

Fig. 2. Heat stability of OA kept for 3, 6, 9 and 12 minutes at 270 °C (arithmetic mean of 3 experiments; ± standard error of the mean)

Carry over of OA from green beans into the brew

Similar results (and similar problems) as for roasting were observed for the carry over of green beans into the coffee brew (figure 3). Even in the low OA level range (<20 µg OA/kg coffee) OA is neither destroyed appreciably by the roasting process nor is it retained in the ground roasted coffee during brewing since the levels found in green coffee beans and in the corresponding brew remained almost the same and no statistically significant difference could be seen. However, the destruction that would have had taken place to detect significant differences between the green

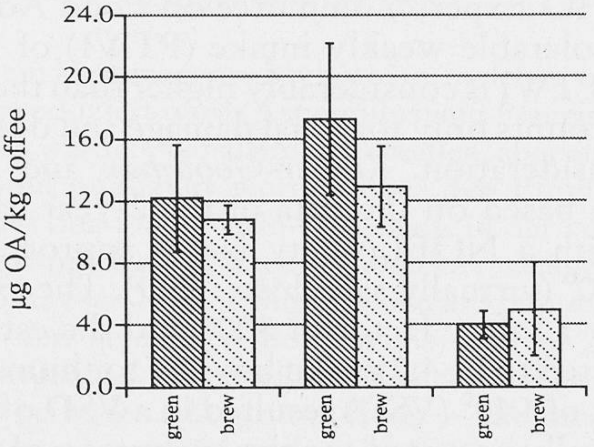

Fig. 3. Carry over of OA from green beans into the brew of 3 commercial samples (the OA amounts are calculated based on green bean weight) (arithmetic means of 4–6 samples; ± standard error of the mean)

coffee and the corresponding brew would have had to be over 52%, 64% and 74% respectively in these three experiments. Since the inhomogeneity in these low contaminated samples is enormous and only a small number of analyses was carried out, it can, based on these data, only be stated that more than 25–50% of the OA, detected in green coffee, is – after roasting – found in the brew. Also *Tsubouchi* et al. (7) reported that OA detected in roasted coffee could be eluted completely into the brew, thus corroborating the assumption that a high percentage (50% or more) of OA found in green coffee is transferred into the coffee brew.

OA in the brew of commercial roasted coffee beans

Based on these results brews of 40 roasted coffee samples from the Swiss retail market were analysed. In 16 samples (= 40%) OA was detected in the range of 1.0–7.8 µg OA/kg coffee (detection limit: 1.0 µg OA/kg coffee). The mean of all samples is 1 µg OA/kg coffee. The higher percentage of positive samples in our study compared to the study of *Tsoubuchi* et al. (9) is due to the lower detection limit, as only 10% of the samples in our study had concentrations >2 µg OA/kg coffee.

Risk assessment

The daily intake of OA via food was estimated to be about 80–100 ng/day/person (11, 12). The daily intake of OA via coffee was calculated based on the preliminary results presented here to be about 25 ng (coffee contamination: 1 µg OA/kg coffee; assumed coffee consumption: 25 ng corresponding to 3–4 cups). Thus the estimated daily intake via food and coffee is about 125 ng/day/person or 2 ng/kg bw/day. This indicates that coffee consumption can contribute significantly to the OA intake of humans.

The Joint FAO/WHO Expert Committee on Food Additives (13) established in 1991 a provisional tolerable weekly intake (PTWI) of 112 ng/kg bw/week or 16 ng/kg bw/day. This PTWI is considerably higher than the estimated daily intake, however, the PTWI accounts only for renal damage and does not take any carcinogenicity data into consideration. *Kuiper-Goodman* and *Scott* (14) calculated a tolerable intake of OA based on the data of the 2-year carcinogenicity study of *Boorman* (2) either with a NOEL/safety factor approach or for a risk of one additional tumor per 10^6 (virtually safe dose, VSD). The experimentally observed NOEL was divided by a safety factor of 5000 and the estimated tolerable intake for humans was calculated to be 4.2 ng/kg bw/day for humans. The linear extrapolation to a life time risk of $1:10^6$ (VSD) resulted in a VSD of 0.2 ng/kg bw/day. The VSD approach is generally accepted for the evaluation of genotoxic carcinogens. Since OA is only – if at all – weakly genotoxic this latter figure is most likely too conservative.

At the moment it is difficult to fully evaluate the carcinogenic risk of human exposure to OA since the carcinogenic mechanism of OA is not known and no kinetic data for humans are available. If, however, the most conservative approach (as used for the VSD) of a linear high to low dose extrapolation is applied, a cumulative theoretical life time risk for a kidney tumor of $12/10^6$ can be calculated from the preliminary data of the total daily OA intake of ca. 2 ng/kg bw. This figure can be compared to the cumulative life time kidney tumor incidence for men and women in Switzerland which is $1.3/10^2$ and $0.6/10^2$ respectively (15). This suggests that OA plays only a minor role among the factors leading to kidney tumors in humans. On the other hand, there are profound species differences in OA kinetics (16) and a longer persistence of OA in humans compared to rats is likely. As long as all these questions are not yet answered and in view of the nephrotoxic and carcinogenic properties of OA the exposure of OA should be kept to a minimum and efforts to reduce the contamination should be undertaken.

Summary

Ochratoxin A (OA) is a carcinogenic mycotoxin which is produced by ubiquitous fungal species (Aspergillus and Penicillium). OA is found in foodstuffs, predominantly in cereals but also in coffee. Inconsistent results have been published regarding the influence of the roasting and brewing process on the OA content in coffee. In this study, ochratoxin A was found in green beans in 13 of 25 analysed samples. Roasting of green coffee beans resulted only in a small reduction of the OA level and OA was also found to be eluted almost completely into the brew. OA was further detected in 16 of 40 analysed brews prepared of coffee samples from the Swiss retail market. These preliminary data suggest, therefore, that regular coffee consumption may contribute significantly to human OA exposure. A preliminary risk assessment based on these data is carried out. However it is difficult to evaluate the carcinogenic risk of human OA exposure as only very limited data on the mechanism and kinetic of OA are available.

Zusammenfassung

Ochratoxin A (OA) ist ein kanzerogenes Mykotoxin, welches vor allem von zwei ubiquitären Schimmelpilzsorten produziert wird (Aspergillus und Penicillium). OA kann häufig in Lebensmitteln nachgewiesen werden, vor allem in Cerealien, aber auch z. B. in Kaffeebohnen. Widersprüchliche Daten sind publiziert worden über den Einfluss des Röstens und der Kaffeegetränkherstellung auf den Ochratoxin-A-Gehalt. In der vorliegenden Studie wurde in 13 von 25 untersuchten grünen Kaffeebohnenproben OA nachgewiesen. Der Röstprozess hatte auf den OA-Gehalt praktisch keinen Einfluss. Auch konnte gezeigt werden, dass OA praktisch vollständig von den gerösteten Bohnen ins Getränk übergeht. In 16 von 40 untersuchten Kaffeegetränkeproben, hergestellt aus Kaffeeproben vom Schweizer Detailhandel, wurde Ochratoxin A gefunden. Aufgrund dieser – allerdings noch unvollständigen Resultate – muss der Schluss gezogen werden, dass der Kaffeekonsum einen gewissen Beitrag zur Belastung des Menschen mit Ochratoxin A leistet. Mit Hilfe der vorliegenden Daten wurde eine erste Risikoabschätzung durchgeführt. Im Moment ist es jedoch schwierig, das

aus der Ochratoxin-A-Belastung resultierende kanzerogene Risiko für den Menschen zufriedenstellend zu beurteilen, da über den Wirkungsmechanismus und die Kinetik praktisch keine Daten vorliegen.

Résumé

L'ochratoxine A (OA) est une mycotoxine cancérigène qui est produite par des espèces fongiques ubiquitaires (p. ex. Aspergillus et Penicillium). OA se trouve dans les aliments, avant tout dans les céréales, mais aussi dans le café. Des résultats contradictoires ont été publiés quant à l'influence des processus de torréfaction et de préparation du café sur la teneur en OA du café consommé. Les résultats présentés ici démontrent la présence d'OA dans 13 des 25 échantillons de café vert analysés. La torréfaction ne réduit pas la concentration en OA de manière significative. On a aussi trouvé que la quasi-totalité de l'OA est éluée dans le café pendant la préparation du café. En outre, on a découvert de l'OA dans 16 des 40 échantillons de café préparés à partir de cafés du marché de détail suisse. Ces données préliminaires suggèrent donc que la consommation régulière de café peut contribuer considérablement à l'exposition humaine en OA. Une estimation préliminaire du risque calculée sur la base des résultats des échantillons analysés est présentée. Cependant, il est difficile de calculer le vrai risque cancérigène qui résulte de l'exposition humaine à l'OA, en particulier parce que la cinétique et le mécanisme de toxicité et de cancérogenèse de l'OA sont pratiquement inconnus.

Literature

1. *van der Merwe, K. J., Steyn, P. S., Fourie, L., Scott, D. B.* and *Theron, J. J.:* Ochratoxin A, a toxic metabolite produced by Aspergillus ochraceus Wilh. Nature **205,** 1112–1113 (1965).
2. *Boorman, G. A.:* Toxicology and carcinogenesis studies of ochratoxin A in F344/N rats. NTP Technical Report **NTP TR 358** (1989).
3. *Cantafora, A., Grossi, M., Miraglia, M.* and *Benelli, L.:* Determination of ochratoxin A in coffee beans using reversed-phase high performance liquid chromatography. Rivista Soc. Scienza Aliment. **12,** 103–108 (1983).
4. *Levi, C. P., Trenk, H. L.* and *Mohr, H. K.:* Study of the occurrence of ochratoxin A in green coffee beans. J. AOAC **57,** 866–870 (1974).
5. *Micco, C., Grossi, M., Miragli, M.* and *Brera, C.:* A study of the contamination by ochratoxin A of green and roasted coffee beans. Food Add. Cont. **6,** 333–339 (1989).
6. *Tsubouchi, H., Yamamoto, K., Hisada, K.* and *Sakabe, Y.:* A survey of occurrence of mycotoxins and toxigenic fungi in imported green coffee beans. Proc. Jap. Ass. Mycotox. **19,** 14–21 (1984).
7. *Tsubouchi, H., Yamamoto, K., Hisada, K., Sakabe, Y.* and *Udagawa, S.:* Effect of roasting on ochratoxin A level in green coffee beans inoculated with Aspergillus ochraceus. Mycopathologia **97,** 111–115 (1987).
8. *Gallaz, L.* and *Stadler, R.:* Ochratoxin A im Kaffee. Chem. Mikrobiol. Technol. Lebensm. **4,** 147–149 (1976).
9. *Tsubouchi, H., Terada, H., Yamamoto, K., Hisada, K.* and *Sakabe, Y.:* Ochratoxin A found in commercial roast coffee. J. Agric. Food Chem. **36,** 540–542 (1988).

10. *Studer-Rohr, J., Dietrich, D. R., Schlatter, J.* and *Schlatter, C.:* The occurrence of ochratoxin A in coffee. Food Chem. Toxicol. (1995), in press.
11. *DFG:* Ochratoxin A, Vorkommen und toxikologische Bewertung. Deutsche Forschungsgemeinschaft, DFG (1990).
12. *Rühl, C., Weber, R.* and *Jiao, Y.:* Ochratoxin-A-Aufnahme mit Cerealien. Tätigkeitsbericht Bundesgesundheitsamt 1991, 214–215 (1992).
13. *WHO:* Evaluation of certain food additives and contaminants. Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series **806,** 29–31 (1991).
14. *Kuiper-Goodman, T.* and *Scott, P. M.:* Risk assessment of the mycotoxin ochratoxin A. Biomed. Environ. Sci. **2,** 179–248 (1989).
15. IARC: Cancer incidence in five continents. IARC Scientific Publications No. 120, 767–769 (Switzerland, Zürich), Lyon, 1992.
16. *Hagelberg, S., Hult, K.* and *Fuchs, R.:* Toxicokinetics of ochratoxin A in several species and its plasma-binding properties. J. Appl. Toxicol. **9,** 91–96 (1989).

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