# *CHAPTER 5*

# Survey of ochratoxin A content in coffee on the South African retail market

The preliminary results of a survey on the OTA levels in coffee on the South African retail market are communicated in this chapter. This survey was supported financially by the MRC and will be concluded in 2000 as part of the candidate's post-doctoral studies at the University of Stellenbosch. The methodology development of some of the work described in this chapter is reported in **Chapter 10**. The purpose of this study was to apply the analytical expertise developed on OTA to the occurrence of OTA in South African coffee samples.

*Contribution made by the candidate*

The candidate was responsible for the design, planning and conducting of the all the experiments, as well as the interpretation of the results.

# Survey of ochratoxin A content in coffee on the South African retail market


## ABSTRACT

Soluble coffee and roasted coffee beans were purchased on the local market and analysed for OTA using aminopropyl- solid phase extraction column clean-up and quantitation with reversed phase HPLC. Preliminary results suggest that the levels of OTA in coffee on the South African market are slightly higher than on the European market. The levels varied between undetectable to 40 ng OTA per gram coffee with the highest levels found in some of the less expensive instant coffee. The average level of ochratoxin A (OTA) for all the coffees analysed was 6 ng/g.


**Keywords**: Coffee, ochratoxin A, amino-solid phase extraction columns, South Africa

## INTRODUCTION

Ochratoxin A (OTA) is a nephrotoxic and carcinogenic mycotoxin produced by *Aspergillus ochraceus* and *Penicillium verrucosum* as well as some isolates of *A. niger, A. terreus* and *A. carbonarius* (CODEX, 1999 and references cited therein). It is a common contaminant in various foods and feeds including coffee, wine, milk, beer, maize and wheat. The International Agency for Research on Cancer (IARC, 1999) has classified OTA as a possible human carcinogen based on its carcinogenic and nephrotoxic effects on experimental animals and its possible role in Balkan endemic nephropathy. The Joint Expert Committee on Food Additives (JECFA) evaluated OTA at its 44th meeting in 1995 and established a Provisional Weekly Intake of 100 ng/kg body weight, which corresponds to approximately 14 ng OTA per kg body weight per day. In 1998 the European Commission's Scientific Committee for Food (SCF) concluded that OTA exposure should be kept to a minimum and that the tolerable daily intakes should be at the lower end of the range of 1.2 to 14 ng/kg which have been estimated by other bodies (European Commission, 1998).

Contradictory reports have been published in the literature on the decomposition of OTA during the roasting process of coffee (Gallaz and Stalder, 1976; Tsubouchi *et al.*, 1988): The data range from almost none to almost complete reduction (van der Stegen *et al.*, 1997).

Although OTA is known to contaminate coffee since the 1970's (Levi *et al.*, 1974), no report has appeared to our knowledge on the OTA content in coffee on the South African retail market. The aim of this study was to determine the levels of ochratoxin in coffee consumed by the South African public and to develop a method for the determination of ochratoxin in coffee, without using the relatively expensive immunoaffinity column cleanup step (Pittet *et al.*, 1996).

## MATERIALS AND METHODS

### *HPLC*

An HP 950 HPLC fitted with an quaternary pump, and HP 1050 fluorescence detector was used with an excitation wavelength of 250 nm and an emission wavelength of 454 nm. A $C_{18}$ -reversed phase column (4.6 mm × 150 mm, 5 μm, Discovery, Supelco, Bellefonte, PA, USA) fitted with a $C_{18}$ guard cartridge (Spherisorb ODS-2, Supelco), mobile phase of methanol/water/acetic acid (60:50:2) and flow rate of 1 ml/min was employed for the analyses.

### *Chemicals*

HPLC grade methanol and acetic acid (Merck) were used for HPLC analysis and chemically pure methanol, acetic acid, hydrochloric acid and chloroform were used for extractions, cleanup steps and derivatisation of OTA to its methyl ester. $NH_2$-LC (aminopropyl, 500 mg, 3 ml sample capacity) solid phase extraction columns were purchased from SUPELCO. OTA standards were isolated from wheat, inoculated with *Aspergillus ochraceus* (MRC 10582, CSIR, Pretoria) using the method of Stander *et al.*, (2000) (recrystallised from benzene, m.p. 91 °C, literature 90 °C, van der Merwe *et al.*, 1965). N-(5-chloro-2-hydroxybenzoyl)-phenylalanine was synthesised by Mr. B.E. Payne, University of Potchefstroom, South Africa (Steyn and Payne, 1999).

### *Sample preparation*

All the coffees were purchased at the local stores, analyses were done in duplicate and only one coffee of each brand-type was analysed. Coffee samples were kept at –20 °C until they were analysed. Soluble coffee (10 g) was dissolved in methanol/water (1:1), made up to a volume of 100 ml, shaken and left for 24 hrs. The coffee was filtered through fluted filter paper. Roasted coffee beans were ground with a coffee grinder for 2 minutes, and the ground coffee (25 g) were mixed with methanol/water (1:1), made up to a volume of 100 ml, shaken and left for 24 hrs to soak.

### *Clean-up steps*

The extraction columns were conditioned with methanol/water (2 ml, 1:1) and the coffee extract (10 ml) was transferred onto the columns, a flow rate of 1 ml/min was employed. After washing the columns with chloroform (2.5 ml), the ochratoxins were eluted with acetic acid/methanol (4:1, 2 ml). The extracts were evaporated to dryness under a stream of nitrogen. The extracts were then resuspended in methanol (250 µl) containing N-(5-chloro-2-hydroxybenzoyl)-phenylalanine (1.16 µg/ml) as internal standard and injected as 50 µl aliquots onto the HPLC.

### *Confirmation of identity*

The remains of the OTA-containing samples (HPLC evidence) following HPLC-analysis were dried under a stream of nitrogen in the HPLC vials. A mixture (200 µl) of concentrated hydrochloric acid (10 µl) in methanol (10 ml) was added to the vials and left at 35-40 °C for 12 hours (Li *et al.*, 1998). Methanol (200 µl) was added to each of the vials, and samples (50 µl) were injected onto the HPLC. The emergence of the OTA methyl ester peak at a retention time of ±32 minutes and the disappearance of the OTA peak at ±21 minutes on the HPLC-chromatogram confirmed the presence of OTA in the sample.

## PRELIMINARY RESULTS

**Table 1**: Results of the screening of OTA in coffee

| Type of coffee | Number of samples | Range (ng/g) | Mean (ng/g) |
|---|---|---|---|
| Ground coffee (mixture of coffee and chicory) | 4 | ≤ 15.3 | 6.8 |
| Ground coffee (pure) | 4 | ≤5.1 | 3.0 |
| Roasted coffee beans | 4 | ≤9.4 | 5.6 |
| Soluble coffee (mixture of coffee and chicory) | 10 | ≤40.4 | 8.1 |
| Soluble pure coffee | 2 | ≤2.3 | 1.6 |
| Decaffeinated coffee | 4 | ≤10.1 | 5.5 |

The recovery in spiked soluble coffee was 98 ± 3 % (±RSD, n = 6), but the recovery proved to vary from one sample of coffee to the other. The detection limit of the HPLC method was 0.5 ng. New linear calibration curves were constructed every day with correlation coefficients of 0.998 or better. Samples with levels of OTA lower than the detection limit were taken as 0.5 ng/g for the purpose of calculating the averages.

## DISCUSSION

The distribution of OTA, especially in the coffee beans, is very heterogenous and led to highly non-reproducible results. Ground coffee and soluble coffee, which were homogenised to an extent by grinding and by liquid extraction, respectively showed less variation. Although the LC-$NH_2$ columns retained the ochratoxins effectively, the active sites of the packing material were sometimes saturated by other compounds present in the coffee, which caused lower recoveries in some of the coffees. Possible solutions to circumvent this problem are to fill the top of the columns with Celite or to use columns with larger quantities of packing material or to reduce the sample volumes sent through the columns. The performance of the HPLC apparatus varied occasionally presumably due to variation in laboratory temperatures. The preliminary results (See **Table 1**) show higher OTA levels in the coffees on the South African market than the levels of OTA in European coffees (van der Stegen *et al.*, 1997).

It is of importance to note that these findings constitute the first report on the natural occurrence of OTA on a food commodity in South Africa, despite the early discovery of OTA by the South African group, van der Merwe *et al.* (1965).

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