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Effect of operating conditions on ochratoxin A extraction from roasted coffee

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Running title: OTA Extraction in Roasted Coffee

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Abstract

Operating conditions affect ochratoxin A (OTA) extraction from roasted coffee. The OTA content found in the beverage can thus be greater than that found in the roasted coffee used to prepare it. Three extraction parameters were studied for roasted coffee : type of extraction solvent (alkaline, neutral, acid), temperature (ambient temperature/23°C, 60°C and 85°C) and extraction time (5, 20, 30, 40, 50, 60 and 80 min). The alkaline solvent that is used in the method recommended by the European Union, extracted OTA better, but a maximum content was obtained at 60°C after 50 min. At least a 100% improvement in extraction was obtained when compared to the EU usual quantification method that is carried out at ambient temperature. It turned out that the OTA extraction parameters for roasted coffee, as defined by that method, were not optimum and needed to be modified. These results were verified in double extraction experiments showing that OTA is not completely extracted by this method. Confirmation was obtained by comparison of extraction methods on several commercial samples of roasted coffee.

Keywords

Roasted coffee; OTA extraction; ochratoxin A

Introduction

Ochratoxin A (OTA) is a mycotoxin produced by moulds of the genera *Aspergillus* and *Penicillium.* In coffee, the OTA-producing strains most frequently found are *Aspergillus niger*, *Aspergillus carbonarius* and *Aspergillus ochraceus (*Frank 2001; Joosten et al. 2001; Suàrez-Quiroz et al. 2004). OTA has nephrotoxic, immunotoxic, teratogenic and carcinogenic effects (Höhler et al. 1998; Pfohl-Leszkowicz and Castegnaro 1999). Its existence in coffee was reported for the first time by Levi et al. (1974). Since then, a great deal of work has been undertaken to study what happens to the toxin during technological processing of coffee, such as roasting and beverage preparation (Viani 1996; Blanc et al. 1998; Van der Stegen et al. 2001). Some work has indicated a higher OTA content in the beverage (prepared hot) compared to that found in the roasted coffee used for beverage preparation (Tsubouchi et al. 1987; Studer-Rohr et al. 1995; Suàrez-Quiroz et al. 2005,). As the usual method used to quantify OTA in roasted coffee (European Union method prEN 14132: 2002 E) recommends extraction at ambient temperature, it was important to study how temperature and duration influenced OTA extraction. The purpose of this work was therefore to describe the effect of different factors (pH, temperature, time) that may affect the OTA extraction in roasted coffee.

Material and Methods

Coffee

The experiments were performed on samples of commercially available roasted, ground coffee.

OTA extraction and quantification parameters

Extraction was carried out in an alkaline solvent (pH 9,4) consisting of methanol and sodium bicarbonate at 3% (20/80; v/v), in a neutral solvent (pH ≈ 6,5) consisting of methanol and distilled water (20/80; v/v) and in an acid solvent (pH 4,3) consisting of methanol and Titrinorm® pH4 buffer (20/80; v/v) (VWR Prolabo, Fontenay sous Bois, France). Extraction was carried out either at ambient temperature (23°C), or in a moderately (60°C) or more heated medium (85°C), with extraction times of 5, 20, 30, 40, 50, 60 and 80 min. Extraction at 85°C required the use of a refrigerant to limit losses through evaporation, and pumice stones for stirring during boiling.

The basic OTA quantification method used was that published by Pittet et al. (1996) for soluble coffee, an adaptation of the method developed by Nakajima et al. (1990). The sample of roasted coffee (10 g) was extracted at ambient temperature for 30 min with 100 ml of alkaline solvent. For that study and all the extraction variants, the filtered extract (5 ml) was diluted in 40 ml of alkaline phosphate buffer (PBS) and the mixture was purified on an immuno-affinity column (Ochraprep®, Rhône Diagnostics, Glasgow, UK). The toxin was eluted from the column with 3 ml of methanol and the eluant was evaporated till dry in a stream of nitrogen at 70°C. The dry extract was re-suspended in 1 ml of mobile phase consisting of distilled water, acetonitrile and acetic acid (51/48/1; v/v/v). Quantification was carried out by HPLC (Shimadzu LC-10ADVP, Japan) with fluorimetric detection. The operating conditions were as follows: 100 µl injection loop, C18 reverse phase HPLC column, ODS particle size 5 µm (Lichrospher 50DS2, Interchim, Montluçon, France) with identical precolumn, thermostatically controlled at 35°C, isocratic flow of 1ml/min, excitation wavelength of 333 nm and emission wavelength of 460 nm. The contents were calculated from a calibration curve established from a standard (1 000 ng ml^{-1} ; ref PD 226 R. Biopharm

Rhône Ltd, Glasgow, UK).

Each extraction and quantification was performed respectively two and three times and the results presented in the form of mean and standard deviation. The limit of quantification of the method is 0.03 µg kg^{-1}.

Results and discussion

Extraction in an alkaline medium

Figure 1 shows the curves for OTA extraction in an alkaline medium, depending on the temperature and extraction time, for a first batch of coffee. The extraction method recommended by the European Union was carried out at ambient temperature for 30 min. OTA quantification by that method gave a content equal to 10.5 ± 0.3 µg kg^{-1}. At the same temperature, other extraction times gave no change in the amount of OTA quantified. However, when extraction was carried out at 60°C, it was clearly improved and then depended on the extraction time. The extraction curve passed through a maximum (31.0 ± 0.3 µg kg^{-1}) at 50 min. A decrease then set in, which was may be linked to OTA degradation or more probably complexing of OTA. Compared to extraction at ambient temperature, a maximum extraction improvement of 195% was obtained. At 85°C, OTA content decreased immediately and continually in line with the heating time. That decrease was a sign of OTA degradation or complexation, which was immediate and probably became preponderant compared to the gain in extraction.

[Insert Figure 1 about here]

Extraction in a neutral medium

The curves for OTA extraction in a neutral medium depending on the temperature and extraction time are shown in Figure 2 for the same batch of coffee. As in the alkaline medium, OTA extraction in a neutral medium at 23°C gave results that remained almost constant, irrespective of the extraction time, and which were almost identical (10.3 ± 0.5 µg kg^{-1}), the difference in extraction falling within the sensitivity limit of the method (0.03 µg kg^{-1}). Consequently, at ambient temperature, there was no significant difference depending on whether extraction was carried out in an alkaline or neutral medium. At 60°C, we found a gradual and continual rise in OTA content in line with the extraction time. After 80 min, the content extracted was 24.4 ± 0.2 µg kg^{-1}. The extraction improvement was therefore 137% compared to ambient temperature, but the amount extracted was smaller than that obtained in an alkaline medium. At 85°C, the quantities extracted increased in line with the extraction time, up to 50 min. At that extraction maximum, the OTA content was 23.1 ± 0.1 µg kg^{-1}, which was greater than for extraction at ambient temperature, but was still below the maximum obtained in an alkaline medium.

[Insert Figure 2 about here]

Extraction in an acid medium

Figure 3 gives the curves for OTA extraction in an acid medium for the same batch of coffee. Extraction at ambient temperature gave quite constant values with a maximum value of 6.4 ± 1.1 µg kg^{-1}. Heating improved the quantities extracted, but the values remained below those obtained with the alkaline and neutral solvents under the same conditions.

[Insert Figure 3 about here]

OTA extraction in a neutral or acid medium did not therefore show any particular improvement compared with that obtained in an alkaline medium. The best extraction rate was obtained with the alkaline solvent at 60°C after 50 min. In order to confirm the fact that extraction at ambient temperature was incomplete, a double extraction assay was performed on a second batch of coffee.

Double extraction

The residue resulting from OTA extraction by the usual method (ambient temperature, i.e. 23°C for 30 min) was recovered and re-quantified under optimum conditions, i.e. at 60°C for 50 min. The OTA content found for the first extraction was 16.5 ± 0.2 µg kg^{-1} (higher content than for the first batch of coffee), whereas that found in the residue after the second extraction was 9.1 ± 0.1 µg kg^{-1}, i.e. a total of 25.6 µg kg^{-1}. Direct extraction at 60°C gave 26.1 ± 0.6 µg kg^{-1} for the same batch. These results show that OTA extraction conditions at 23°C were not optimum and largely underestimated the OTA content. Both the temperature and the extraction time were inadequate.

Verification of the method on several samples of roasted coffee

Twenty-four different brands of roasted, ground coffee were bought commercially and quantified using the method recommended by the EU (alkaline solvent at 23°C for 30 min) and the alternative method proposed (alkaline solvent at 60°C for 50 min). Table 1 gives the results of a comparison between the two extraction methods on samples of roasted coffee.

[Insert Table 1 about here]

An improvement in OTA extraction, ranging from 100 to 200%, was obtained with the new method for all the coffee samples contaminated with OTA. The degree of improvement in the extraction rate obtained with the new method varied from sample to sample. That variation could be explained by the type of coffee (arabica, robusta, or blend) and the degree of roasting (light, medium, dark). During roasting, reactions leading to reversible chelation of OTA with other molecules such as proteins can occur (Il'ichev et al. 2002). Figure 4 shows a chromatograms of a commercial sample of roasted coffee, a same spiked sample (with 2, 5 µg kg^{-1} added), and a sample of coffee beverage.

[Insert Figure 4 about here]

The purpose of this study was to explain the increase in OTA content found in a coffee beverage compared to that in the roasted coffee used to prepare it. The increase was due to the operating conditions used. The solvent (pH), temperature and extraction time affected the OTA content obtained. The EU method (alkaline solvent at ambient temperature for 30 min) gave unsatisfactory OTA extraction rates. Alkaline solvent was best for extracting OTA from roasted coffee, provided it was extracted at 60°C for 50 min. An improvement of at least 100% was observed. The results of OTA analysis in roasted coffee or the beverage by the conventional technique therefore need to be treated with caution. As the legislation fixes a tolerable limit of 5 µg kg^{-1}, it would be advisable to revise the analysis conditions.

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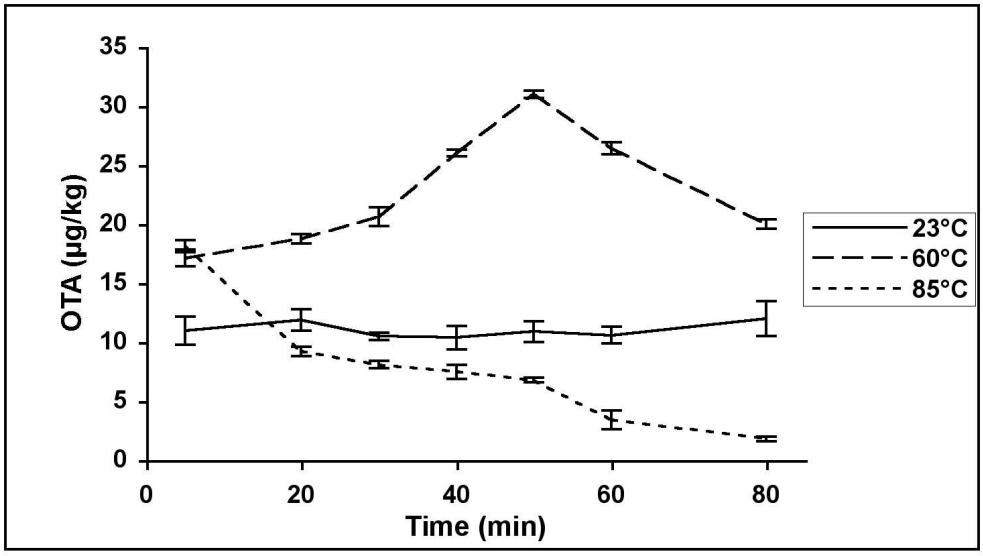
Figure captions

Figure 1. Effect of temperature and extraction time on OTA extraction in an alkaline solvent

Figure 2. Effect of temperature and extraction time on OTA extraction in a neutral solvent

Figure 3. Effect of temperature and extraction time on OTA extraction in an acid solvent

Figure 4. HPLC chromatograms of : commercial sample of roasted coffee, spiked sample with 2.5 µg OTA kg^{-1} added, coffee beverage sample



Effect of temperature and extraction time on OTA extraction in an alkaline solvent
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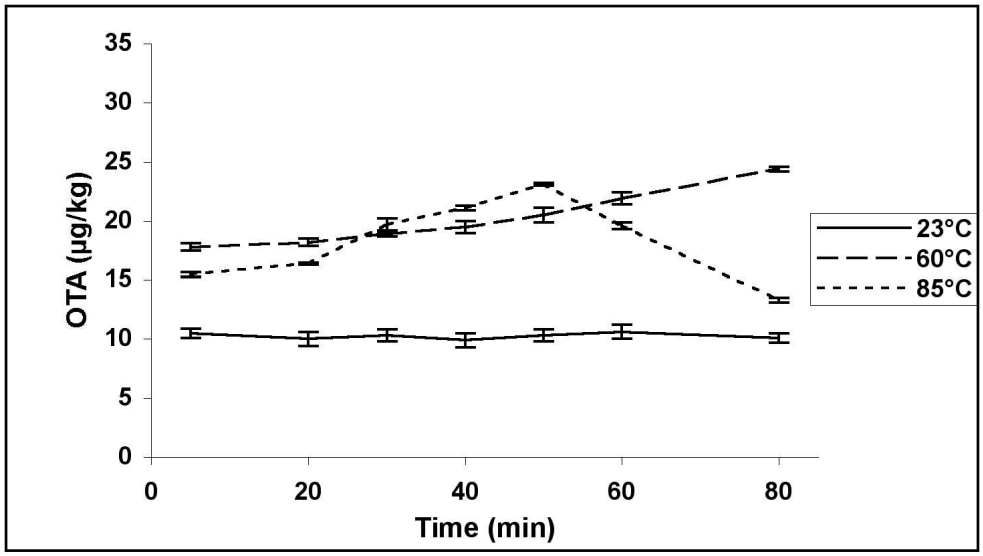

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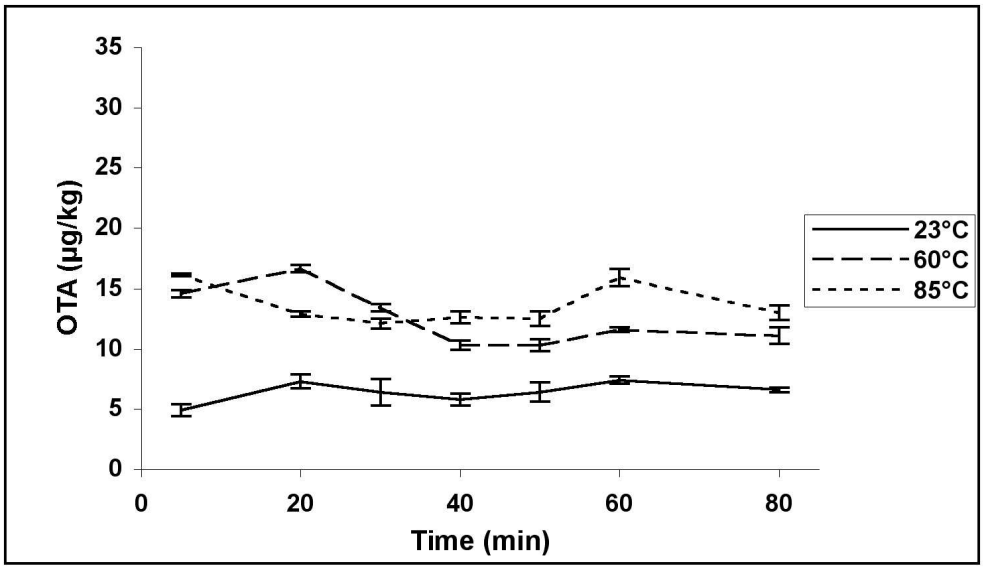

Effect of temperature and extraction time on OTA extraction in an acid solvent
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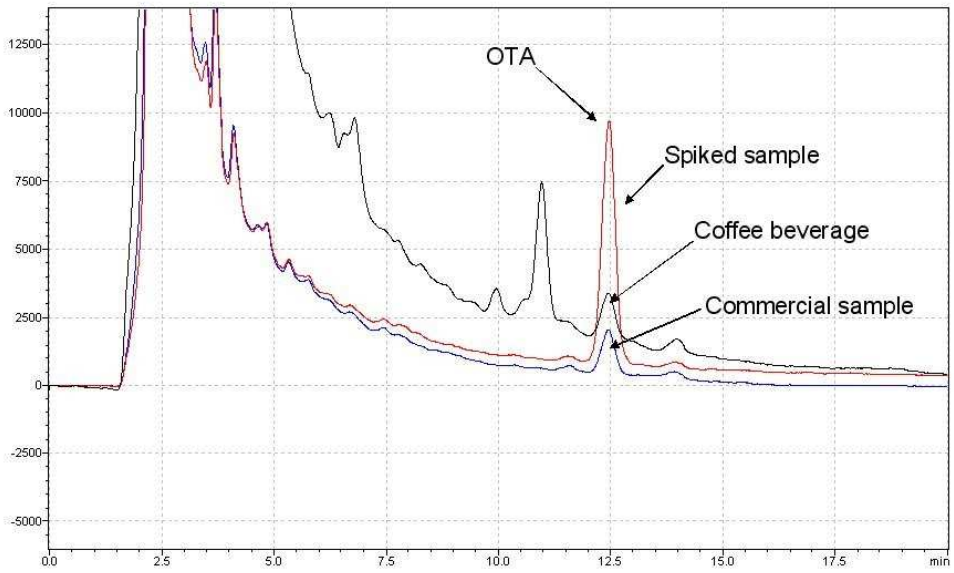

HPLC chromatograms of commercial sample of roasted coffee, spiked sample with 2.5 µg OTA kg-1 added, coffee beverage sample
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