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Influence of roasting and different brewing processes on Ochratoxin A content in coffee determined by HPLC-FLD

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3 1 **Influence of roasting and different brewing processes on the ochratoxin A**
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6 2 **content in coffee determined by HPLC-FLD.**
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26
27 10 **ABSTRACT**
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31 11 A rapid and reliable procedure has been developed for the determination of ochratoxin A (OTA) in
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33 12 green and roasted coffee. The method consists of extraction of the sample with methanol -5%
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35 13 aqueous sodium hydrogen carbonate/1%PEG8000- (20:80), followed by IAC clean up and finally,
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37 14 HPLC determination with fluorimetric detection. Mean recoveries for green and roasted coffee
38
39 15 spiked at different levels ranging from 94 and 105 % were obtained. The limit of determination
40
41 16 (S/N= 3) was 0.032 ng/g and the precision (within-laboratory relative standard deviation) was 6%.
42
43 17 The method described has been used to assess the influence of roasting and different brewing
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45 18 processes on OTA content in commercial lots of green and roasted coffee. The results provided
46
47 19 evidence that roasting led to a significant drop on OTA levels (65-100%). Also the way coffee is
48
49 20 prepared affects the OTA content: brewing using a moka express (Italian coffee) led to a significant
50
51 21 reduction of OTA concentration (50-75%) since hot water stays in contact with coffee for a short
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53 22 time, on the contrary Turkish coffee making (infusion for about 10 min) cause poor reduction in
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55 23 OTA
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24 **Keywords:** brewing methods, coffee, HPLC-FLD, ochratoxin A.

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3 25 RUNNING HEADER: Influence of coffee processing on OTA concentration
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6
7 26 **Introduction**
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10 27 The occurrence of ochratoxin A (OTA) in raw coffee has been reported in various studies since
11
12 28 1974 (Levi et al, 1974; Micco et al, 1999; Nakajima et al, 1997; Tsubouchi et al, 1984; Truckness et
13
14 29 al, 1999). OTA is a mycotoxin produced as a metabolic product of certain fungi, mainly of the
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16 30 genera *Aspergillus* and *Penicillium*. OTA toxicity is mainly a result of interactions with nucleic
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18 31 acids, determining deleterious effects on the protein synthesis and integrity of nucleic acids with
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20 32 consequent damage to the organisms; for these reasons, OTA is considered one of the most
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22 33 powerful natural hepatocarcinogenic agents (Chu, 1991; Galvano, Ritieni, Piva , Pietri 2005). The
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24 34 carcinogenicity of OTA has been evaluated by the IARC in a recent monograph where OTA is
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26 35 classified as Group 2B, possible human carcinogen (IARC, 1993)
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34 37 The EU has fixed legal limits for the occurrence of Ochratoxin A (OTA) in following foods: cereals
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36 38 (5 ng g^{-1}), products derived from cereals (3 ng g^{-1}), wine and grape juice (both 2 ng g^{-1}) dried vine
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38 39 fruit (currants, raisins and sultanas all 10 ng g^{-1}), and from 2005 also roasted coffee (5 ng g^{-1}) and
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40 40 soluble coffee (10 ng g^{-1}) (EU Regulation 123/2005). The growth of mould on coffee beans is
41
42 41 possible if the moisture content of the beans exceeds the accepted standard for an extended time
43
44 42 (Studer-Rohr et al, 1995). An Italian study carried out by Romani et al in 2000 provided evidence
45
46 43 that 106 out of 162 green coffee samples coming from different countries (84 from Africa, 60 from
47
48 44 America, and 18 from Asia) were contaminated by OTA, with concentration ranging from 0 to 48
49
50 45 ppb. In particular, it was possible to verify that, samples from African countries were more
51
52 46 contaminated as compared to samples from other origins in terms of frequency and level of OTA.
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54 47 How the content of the toxin in soluble coffee relate to maximum concentrations in the cup depends
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56 48 of course very much on brewing strength and also on the extraction efficiency (the latter mostly
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58 49 being high). Brewing strengths are quite variable between different countries across Europe and all
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3 50 around the world. During the last 30 years many studies have discussed the effect of roasting and
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5 51 brewing procedures on OTA content in coffee (Blanc et al, 1998; Cantafora et al, 1983; Gallaz and
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8 52 Stadler 1976; Levi et al, 1974; Romani, 2003; Studer-Rohr et al 1995; Tsubouchiet al, 1987; van
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10 53 der Stegen et al, 2001) and, as Pittet observed in 1999, many inconsistencies were found since
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12 54 OTA reduction ranging from 0 to 100% during roasting or brewing were reported. These
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15 55 contradictions were attributed to variability in the types of coffee contamination and different
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17 56 roasting conditions (van der Stegen et al, 2001).

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22 58 This study aims to give further insight into the effect of roasting (200 C° for 3 min) and brewing on
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24 59 the reduction of OTA. The most diffused procedures to brew coffee have been studied

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26 60
- 27 61 - Infusion: the most common method is to put the ground coffee in a cup, pour hot water over
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29 62 an let it stand to cool and allow the grounds to sink to the bottom. This is the traditional way
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31 63 to make coffee in Finland and Norway, which have the highest coffee consumption per
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33 64 capita in the world (<http://www.nationmaster.com>). Also the so-called Turkish coffee is
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35 65 based on the principle of brewing coffee by infusion
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37 66
 - 38 67 - Pressure: using a moka express which is a stovetop made of aluminium; it is based on the
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40 68 principle of preparing coffee by steam. Due to the higher pressures involved, the mixture of
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42 69 water and steam reaches temperatures well above 100 °C, causing a more efficient
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44 70 extraction of the coffee components, and resulting in a much stronger brew when compared
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46 71 to that obtained by drip brewing or percolation. It was created in Italy by Alfonso Bialetti in
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48 72 1933. In Italy the moka express is in almost every home. About fourteen billion espresso
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50 73 coffees are consumed each year in Italy and Italians consume approximately 3.2 kg of coffee
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52 74 per capita (<http://www.nationmaster.com>).
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60 - Gravity: drip brew, commonly named American coffee, is made by letting hot water drip
onto coffee grounds held in a coffee filter (paper or perforated metal).

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3 75 Over the past few years, the sensitivity of analytical techniques for the detection of OTA has
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5 76 improved. The most diffused technique for OTA analysis in food is liquid chromatography with
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8 77 fluorescence detection (LC–FLD), preceded by sample purification by using immunoaffinity
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10 78 columns (Pittet et al, 1996; Sibanda et al, 2002), but also the use of different types of columns was
11
12 79 described (Ventura et al, 2003). In this study OTA extraction from green and roasted coffee was
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14
15 80 carried out using methanol-5% aqueous sodium hydrogen carbonate /1% PEG8000 (20:80)
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17 81 followed by immunoaffinity clean up; this procedure was compared with the one described by Pittet
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19 82 et al (1996) which is the most reliable and diffused analytical method to measure OTA levels in
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22 83 green and roasted coffee.
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27 85 **MATERIALS AND METHODS**

28 29 86 *Reagent*

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31 87 HPLC grade acetonitrile, water, methanol were purchased from Romil (Cambridge, GB). The OTA
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33 88 standard was purchased from Sigma (Sigma–Aldrich, Milan, Italy). Samples were filtered through a
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36 89 Sartorius PES 0.2 µm membrane (Goettingen, Germany). A standard solution of OTA was prepared
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38 90 from the OTA vial purchased from Sigma. The standard stock solution was made in 4 mL of
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41 91 methanol at 250 µg/mL, and stored at -20 °C. An intermediate standard solution was prepared at 10
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43 92 µg/mL, by diluting 1 mL of stock standard solution with 25 mL of methanol. All of the solutions
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46 93 were stored at -20 °C. The RIDA IACs were obtained from Bio-Pharm (Darmstadt, Germany).
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48 94

49 94 *Apparatus*

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51 95 An Agilent 1100 Series liquid chromatograph equipped with a G1312A binary pump, a G1321A
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53 96 fluorimetric detector, and a Rheodyne 7125 injection valve fitted with a 20 µl loop, a Phenomenex
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56 97 (Terrance, CS, USA) Thermasphere column oven, was used.
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58 98 *HPLC-FLD conditions*

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60 99 The separation of OTA was achieved with a Phenomenex Luna C18 Column (150x4.6 mm, 5µm
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particle size) and a guard column packed with the same material using a mobile phase composed of

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3 101 $\text{CH}_3\text{CN}/\text{H}_2\text{O}/\text{CH}_3\text{COOH}$ (54:44:2, v/v) at a flow rate of 1mL/min; the column temperature was
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5 102 30C°. Fluorescence excitation and emission wavelengths for OTA detection were 334 and 460 nm,
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7
8 103 respectively; an adequate signal-to-noise ratio was obtained with a photomultiplier gain of 14 and a
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10 104 response time of 4 sec.

12 105 *Samples*

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15 106 Samples were cleaned, roasted, cooled, ground, and packaged in a Sicilian firm (ZiCaffè, Marsala,
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17 107 Italy). Bags of green coffee beans are machine-opened, dumped into a hopper, and screened to
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19 108 remove debris. The green beans are then transferred by belt to storage hoppers. From the storage
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21
22 109 hoppers, the green beans are conveyed to the roaster. 240 Kg of green coffee were simultaneously
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24 110 roasted. Roasters operate at temperatures of 200°C for 3 minutes. Experiments were carried out
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26
27 111 using 14 lots of 650 kg of green coffee beans coming from different countries (4 from India, 5 from
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29 112 Vietnam, 2 from Cameroun, 1 from Ethiopia, 1 from Brazil and 1 from Salvador). Nine
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31 113 commercial soluble coffee samples bought in a supermarket in Palermo (Italy) on July 2007 were
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33
34 114 also analysed.

36 115 *Extraction with methanol -5% aqueous sodium hydrogen carbonate/1%PEG8000- (20:80)(A)*

38 116 10 g of grounded roasted or green coffee were mixed with 100 mL of methanol-5%NaHCO₃ / 1%
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41 117 PEG8000 (solution A) and placed in a ultrasound bath at 50°C for 10 min. After this time the
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43 118 mixture was filtered trough filter paper, then 10 mL of the filtrate were transferred in a graduate
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45
46 119 cylinder and diluted to 20 mL with a solution of 5%NaHCO₃ – 1 % PEG8000 and loaded onto the
47
48 120 IAC.

50 121 *Extraction with methanol-3% aqueous sodium hydrogen carbonate (50:50)(B)*

52 122 This extraction method was similar to the one described by Pittet et al (1996). 10 g of grounded
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54
55 123 roasted coffee were mixed with 100 mL of methanol-3% aqueous sodium hydrogen carbonate
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57 124 (50:50) (solution B) and placed in a ultrasound bath for 10 minutes. After this time the mixture was
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60 125 filtered trough filter paper, then 10 mL of the filtrate was transferred in a graduate cylinder and
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diluted to 50 mL with PBS. Aliquots of 20 mL were passed over the IAC.

Coffee Brewing

Infusion: 10 g of grounded roasted coffee were mixed with 100 mL of hot water. After ten minutes the mixture was filtered through filter paper, and extracted with 100 mL of solution A as described. Then 10 mL of the extract was diluted with 10 mL of a solution of 5%NaHCO₃ – 1 % PEG8000 and loaded onto the IAC.

Brewing with an Italian moka pot: 100 mL of water were poured in the boiler, then the funnel-shaped metal filter is inserted. 25 g of ground coffee is thereupon added, and the upper part is tightly screwed on the base. By placing the pot on a heat source, the water is brought close to boiling point creating steam in the boiler. The espresso is created when the steam reaches a high enough pressure: it gradually forces the surrounding water up the funnel through the coffee powder and into the upper chamber, where the coffee is collected. The brew was transferred into a 100 mL graduated cylinder, made up to the mark with water and extracted with 100 mL of solution A as described earlier. Then 10mL of the extract were diluted with 10 mL of a solution of 5%NaHCO₃ – 1 % PEG8000 and loaded onto the IAC.

Drip brew: 100 mL of boiling water were let drip onto 25 g of coffee grounds placed onto a metal filter; the mixture was collected in a 100 mL graduate cylinder. The operation was repeated three times. Then 100mL of the brew were processed as described earlier for OTA extraction.

Immunoaffinity column cleanup.

A volume of 20 mL of diluted extract were loaded onto the immunoaffinity column, at a slow steady flow rate of about 3 mL/min. After washing the column with 5 mL of distilled water, OTA was eluted with 3 mL of methanol. Then the solvent was evaporated to dryness using a rotary evaporator at 60 °C. The residue was dissolved in 500 µl of mobile phase for injection into the liquid chromatograph.

Samples fortification

Recovery experiments were performed by spiking 10 g of blank samples with 100 µl of spike solutions (prepared by appropriate dilution of the stock solution). Spiked samples were left at least 1

h, to allow solvent evaporation prior to extraction. To study the between-day precision of the method, OTA fortified (2 ng/g) samples, were analysed in triplicate for 3 days.

RESULTS AND DISCUSSION

Method

There is an increasing demand for new OTA extraction techniques from coffee, cereals, and many other food matrices, with shortened extraction times and reduced costs which ensures high recovery, precision and low limit of detection (LOD) of the toxin. In 2007 Liazid et al. described the stability of OTA during its extraction using different extraction techniques, including ultrasound-assisted extraction (UAE) and magnetic stirring assisted extraction (MSAE), applied to a model solution. In this study we tested the use of ultrasound for OTA extraction from coffee using two different solvents: methanol - 5% aqueous sodium hydrogen carbonate /1% PEG8000 (20:80) (solution A) and methanol-3% aqueous sodium hydrogen carbonate (50:50) (solution B). The effect of ultrasound is to help penetration of the solvent in the sample matrix and to promote the dissolution of OTA in the extracting solvent (Liazid et al, 2007). **Figure 1** provides evidences that the application of ultrasound at 50 °C for 10 min. ensures good recoveries both using solution A (99.0±0.70%) or solution B (95.0±0.73%) as the extraction solvent from an artificially contaminated coffee spiked with 2 ng/g of OTA. No significant influence of temperature (40, 50 and 60 °C) was found (data not shown). Therefore further experiments were performed by extracting OTA with solution A in a ultrasound bath for 10 min. at 50°C°. The extraction procedure with solution A plus IAC clean-up provided a clean LC–FLD chromatogram for green and roasted coffee (flat baseline, practical lack of impurity peaks) due to IAC selectivity, as can be seen in **Fig. 2**

The results from recovery test (**Table 1**) provided evidence that the extraction of OTA with solution A yielded higher levels of the toxin (by 1-8%) compared to those obtained using methanol-3% aqueous sodium hydrogen carbonate as suggested respectively by Pittet. (1996) and van der Stegen

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3 179 et al. (2001). The higher recoveries obtained in this study may be explained by the use of PEG 8000
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5 180 and higher NaHCO₃ percentage in the extraction and diluting solutions as reported by Visconti et al
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8 181 (1999) in a study concerning OTA extraction from wine. The between- day coefficient of variation,
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10 182 which is very important when establishing routine methods to be used over extended periods, for
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12 183 method A was of 3.6%; precision calculated as RSD% of nine measurements of a spiked sample (2
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14 184 ng/g), was 6%. A calibration curve ($y=3.2997x$, $R=0.9984$) was obtained spiking homogenised
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16
17 185 blank samples with OTA covering the range from 0.05 to 50 ng/g (**Fig. 3**). The limit of detection
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19 186 (LOD) and the limit of quantification (LOQ), calculated as 3- and 10-fold the signal to noise ratio,
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21 187 were 0.032 and 0.107 ng/g, respectively, lower than those found by Pittet et al (1996), van der
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23 188 Stegen et al (2001), Sibanda et al (2002). The method described fits with the requirements of
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26 189 European legislation regarding methods of sampling and analyzing mycotoxins in foodstuffs (EU
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29 190 Regulation n. 401/2006)

31 191 *Effect of roasting on reduction of OTA*

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33 192 Nine samples of commercial coffee, 14 of green coffee from different producing countries and the
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36 193 corresponding ground roasted samples were analysed (**table 2**). Green coffee samples contained
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38 194 OTA amounts ranging from 0.567 to 2.55 ng g⁻¹. Four out of 14 of the corresponding roasted
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41 195 samples had OTA levels lower than the LOD, whereas the other samples had OTA concentrations
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43 196 ranging from 0.089 to 0.935 ng g⁻¹. Four out of 6 of commercial roasted coffee miscellaneous
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45
46 197 presented OTA levels from 1 and 2.2 ppb, the other two samples lower than 0.5 ppb. **Table 2** gives
47
48 198 evidence that roasting procedure at 200 °C for 3 min led to a significant reduction ($t < 0.0001$) in
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50 199 OTA, ranging from 65 to 100%. These results are in agreement with those found by Viani (1996),
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52 200 Blanc et al. (1998) and Stegen et al.(2001) that observed strong OTA reduction after roasting

53 201 *Effect of brewing on reduction of OTA*

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55 202 The effect of moka express brewing (Italian coffee), drip brewing and infusion in hot water
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58 203 (Turkish coffee) on the OTA content was studied. To determine the significance of OTA reduction
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60 204 by brewing coffee, a paired t-test was performed. The results (**Table 3**) gave evidence that OTA in

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3 205 naturally and artificially contaminated coffee containing low amounts of the toxin ($0.1-0.5 \text{ ng g}^{-1}$)
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5 206 and brewed using a Moka express, was reduced by 51-57% ($p>0.005$), stronger OTA reductions
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8 207 (58-75%) in naturally and artificially contaminated samples containing higher levels ($1-12 \text{ ng/g}$) of
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10 208 the toxin were found. Coffee brewing using a moka pot is a very short process (about 2 min.),
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12 209 therefore longer time might be needed to infuse all the toxin into the brew when OTA is present in
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15 210 the grounded beans at high levels. To confirm that the observed OTA reduction was due to an
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17 211 partial solubilization of the toxin into the brew, after moka brewing, OTA extraction was performed
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19 212 again on the residues of naturally contaminated coffee samples containing 0.5, 0.93 and 2.1 ng/g of
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21
22 213 OTA respectively, and recoveries ranging from 45 to 71% were obtained, suggesting that the
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24 214 greatest part of the toxin originally present in the coffee was not extracted during moka brewing.
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26 215 Similar results were obtained for drip brewing (reduction ranging from 71 to 73%), which might
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29 216 also be explained by the short duration of the process. An average OTA reduction of 20 % was
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31 217 found after infusion of grounded coffee in hot water for 10 min (Turkish coffee); in this case the
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34 218 time spent in contact with water is longer therefore higher amounts of the toxin were solubilized
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36 219 into the brew.

OTA behaviour during heating

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40 221 To gain information about the behaviour of OTA in hot water and to assess a possible interaction of
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43 222 the toxin with the substrate during coffee brewing, the following experiments were undertaken: a)
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45 223 kinetics of OTA in pure hot water (water temperature was kept at $95-96^\circ\text{C}$ over time) b) reduction
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48 224 of OTA by brewing coffee in hot water over time (the temperature was kept at $95-96^\circ$ over time)
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50 225 using a naturally contaminated sample with 2.1 ng g^{-1} c) reduction of OTA by brewing coffee in
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52 226 hot water (water at 95°C was poured onto grounded coffee and it was let to cool during the
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54
55 227 experiment; after 45 min. the temperature was 40°C). **Figure 4** shows that OTA content in pure
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57 228 water (a) after 2 and 5 min. was reduced by 5-15%; after 10 min OTA decrease was of 23%,
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59 229 whereas by prolonging heating up to 45 min OTA levels decreased by 44-61%. Two different
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230 explanations are available for this reduction: isomerization at the C-3 position into another

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3 231 diastereomer, and thermal degradation (Boudra et al, 1995; Studer-Rohr et al, 1995). In this case the
4
5 232 results obtained revealed first-order reaction ($y=-0.024x$, $R^2= 0.918$), whereas a more complex
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7
8 233 kinetics was observed in cases b and c. OTA reduction of 70 % was observed by heating coffee 2
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10 234 minutes in 95 C° water (b), in agreement with results obtained by brewing coffee with a moka
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12 235 express; prolonging heating time up to 10 min a greater amounts of the toxin is transferred into the
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15 236 decoction and OTA decrease ranged between 23-30%. By prolonging coffee infusion up to 45 min,
16
17 237 OTA amounts decreased by 73%; the higher OTA reduction observed after 10 min respect to the
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19 238 experiment in pure boiling water could be explained by a possible interaction of the toxin with the
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21
22 239 coffee-substrate (Il'ichev, Perry, Rümer, Dockal, Simon, 2005; Suárez et al, 2005). A different
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24 240 behaviour of OTA was observed brewing coffee in the Turkish mode (c) since after 10 min the
25
26 241 reduction of the toxin kept constant (25-29%). This suggested that the temperature might influence
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28
29 242 OTA transformation and its interaction with the substrate.

30 31 243 **Nutritional considerations**

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33 244 Coffee samples of this study represent a poor alimentary source of OTA, particularly if it is brewed
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36 245 using a moka-expresso, according to the Italian style. Considering a mean level of OTA in coffee of
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38 246 1 ng/g and the Tolerable Daily Intake for OTA of 300 ng (for a 60 kg b.w. adult), according to this
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41 247 study, a 20 mL cup of moka-express prepared as described above, provides about 1.25-2.5 ng of
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43 248 OTA, which represent maximum the 0.8 % of the TDI. Whereas a 100 mL cup of Turkish coffee
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45 249 provides about 20 ng of the toxin, which accounts for the 6.7 % of the TDI.

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48 250 Even though this study shows that a moderate consumption of coffee, both prepared in the Italian or
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50 251 Turkish mode provides OTA intakes well within the safety limits, since significant reduction of the
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52 252 toxin was observed during roasting and brewing processes, it is of main importance to prevent green
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55 253 coffee contamination by applying all the preventive measures along the supply chain.

56 57 254 **Acknowledgements**

58
59 255
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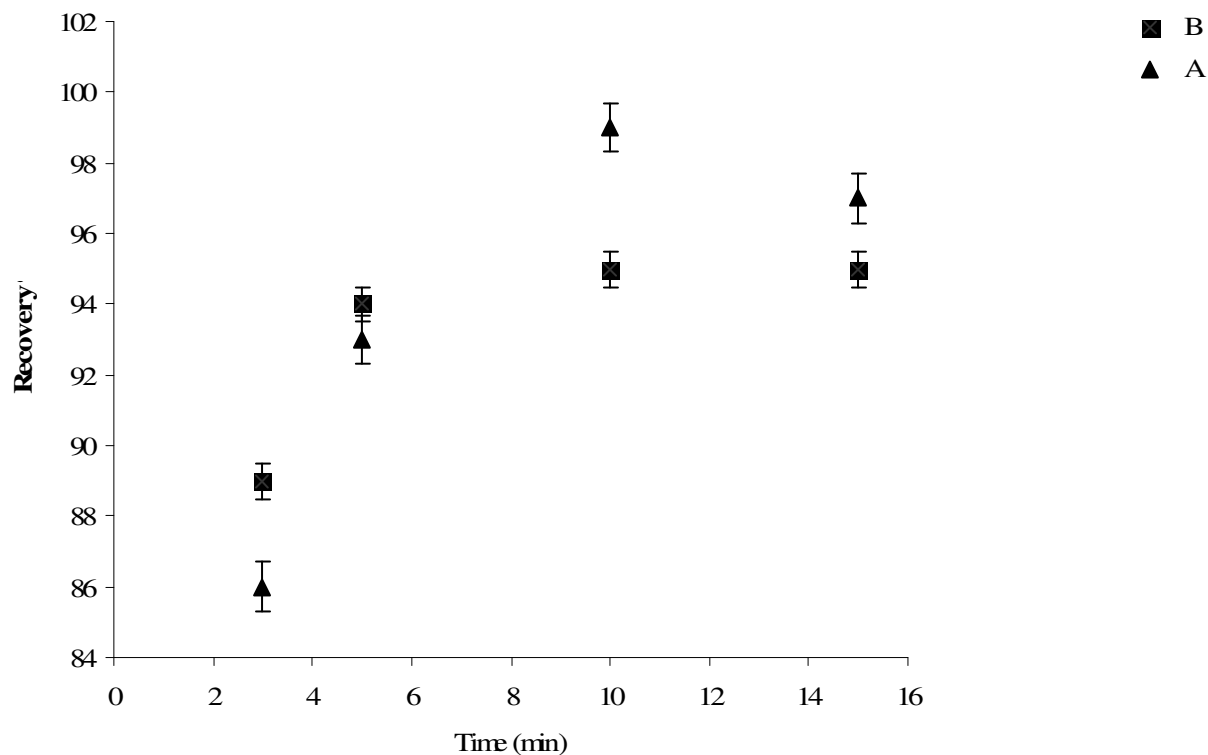


Figure 1 Effect of ultrasound application time at 50 °C on OTA extraction from an artificially contaminated coffee spiked with 2 ng/g of the toxin. Each point represents a mean of three replicates, the RSD% of the measures was of 0.75%.

A: extraction with methanol -5% aqueous sodium hydrogen carbonate/1%PEG8000- (20:80)

B: extraction with methanol -3% aqueous sodium hydrogen carbonate (50:50)

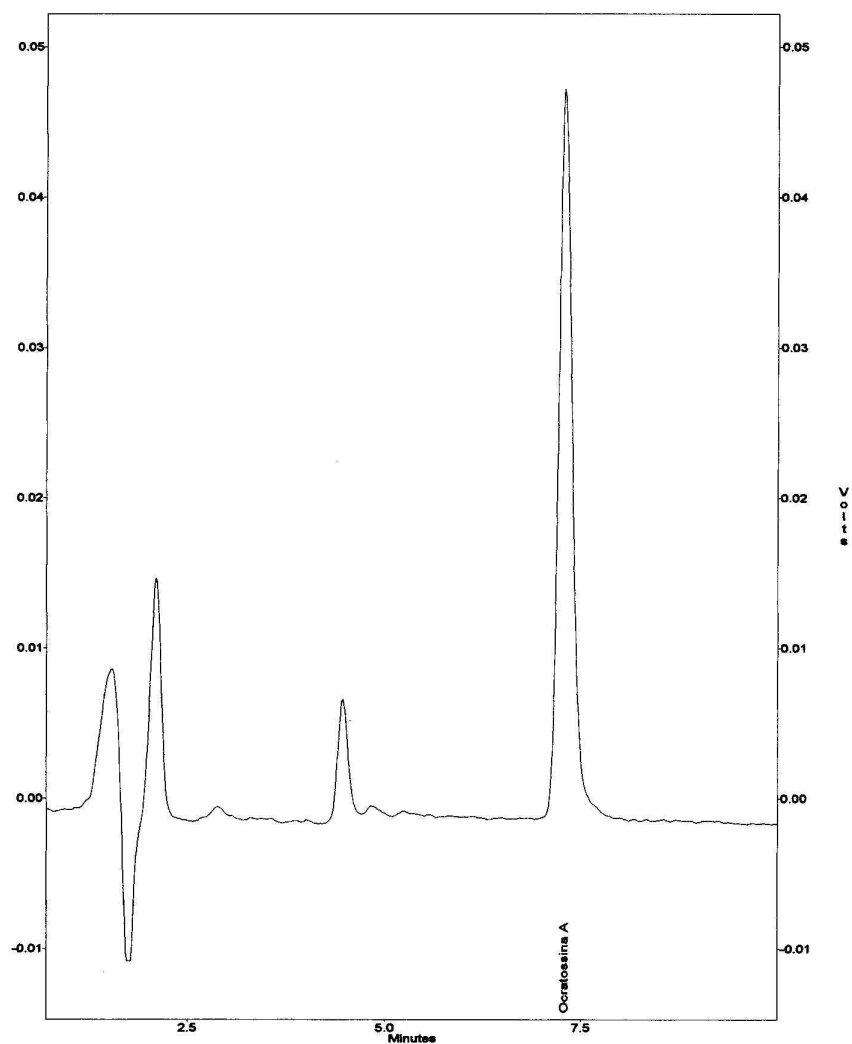
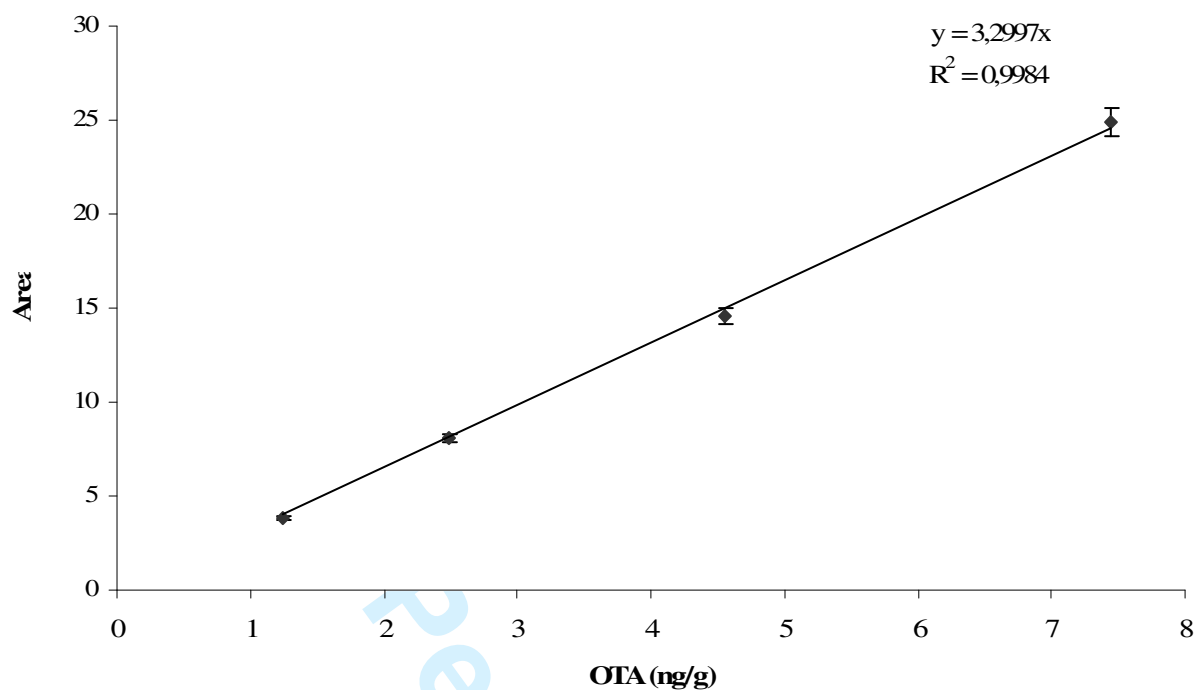


Fig. 2. LC-FLD chromatogram obtained by extraction of roasted coffee with solution A, filtration and IAC clean-up of a OTA free sample fortified with 2ng/g of the toxin. Chromatographic conditions: column, Phenomenex Luna C18 reversed-phase column (150mm × 4.6 mm, 5 μm particle size); column temperature, 30 °C; mobile phase, acetonitrile–water–acetic acid (54:44:2, v/v/v); flow-rate, 1.0 ml/min; injection volume, 20 μl. Excitation wavelength, 333 nm; emission wavelength, 460 nm.



28 **Figure 3** Calibration curve. Each point is the mean of three replicates, the RSD% of the measures is
29 of 3.0%.
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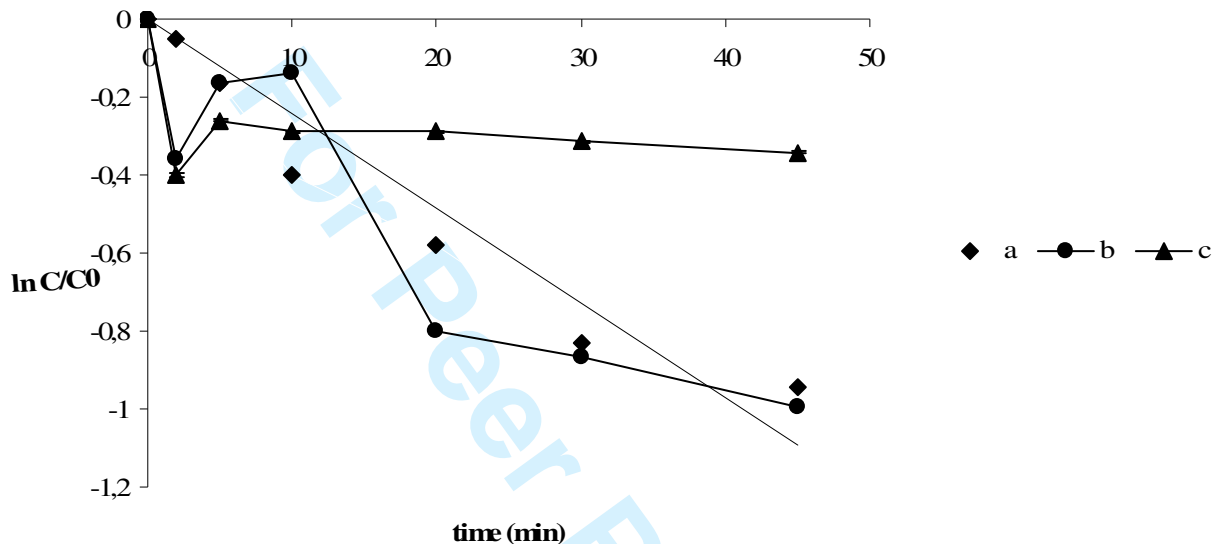


Figure 4 Kinetics of OTA disappearing during heating in different conditions*: a) kinetics of OTA in pure hot water (water temperature was kept at 95-96°C over time) b) reduction of OTA brewing coffee in hot water over time (the temperature was kept at 95-96°C over time) using a naturally contaminated sample with 2.1 ng/g c) reduction of OTA brewing coffee in hot water (water at 95°C was poured onto grounded coffee and it was let to cool during the experiment).

*Each point represents the mean of three replicates. The RSD of the measures was of 1% for curve a and 2% for curve b and c

Table 1 Mean recoveries obtained for OTA extraction with methanol-5% aqueous sodium hydrogen carbonate/1%PEG8000 (20:80) (A) and extraction with methanol-3% aqueous sodium hydrogen carbonate (50:50) (B) from blank and naturally contaminated samples

<i>SAMPLE</i>	<i>OTA (ng/g)</i>	<i>OTA added (ng/g)</i>	<i>Recovery % (A)</i>	<i>Recovery % (B)</i>
ROASTED	nd	0.5	94.0	93.1
	nd	2.0	95.0	93.0
	nd	4.0	96.5	95.5
	nd	10.0	98.4	91.0
	0.930	2.0	95.0	95.0
	0.930	4.0	99.5	95.1
	0.930	10.0	102.0	94.3
GREEN	nd	2.0	105.1	98.0
	nd	5.0	99.0	95.1
	nd	15.0	96.0	92.4
	2.2	2.0	98.0	92.0
	2.2	5.0	98.1	94.0
	2.2	15.0	95.2	94.3

Table 2 Range levels of OTA in green and roasted coffee samples from different origins

<i>SAMPLE</i>		<i>Green OTA (ng/g)</i>	<i>Roasted OTA (ng/g)</i>	<i>Reduction range%*</i>
<i>INDIA</i>	4	0.567-1.133	nd-0.121	87-100
<i>VIETNAM</i>	5	1.067-2.553	nd-0.930	81-87
<i>CAMEROUN</i>	2	1.077-1.403	0.150-0.497	65-86
<i>SALVADOR</i>	1	1.593	0.108	93
<i>BRAZIL</i>	1	0.703	<0.032	100
<i>ETHIOPIA</i>	1	0.632	0.140	77
<i>Commercial miscellaneous</i>	2	not available	0.3-2.2	-

*Reduction was significant at $t < 0.0001$

Table 3 Effect of brewing on OTA levels in roasted grounded coffee

Type of contamination	n. of samples	OTA levels (ng/g)	% reduction by moka pot	% reduction by drip brew	% reduction by Infusion*
<i>natural</i>	4	0.1-0.5	51-57	55	20-23
<i>natural</i>	4	0.5-1	55-59	62-64	22-25
<i>natural</i>	3	1.5-2.2	63-71	67-68	22-25
<i>artificial</i>	3	0.1-0.5	58-60	54-58	18-22
<i>artificial</i>	3	2-5	59-63	62	19-20
<i>artificial</i>	3	8-12	69-75	71-73	17-20

*Infusion time= 10 min.

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