Effect of two different roasting techniques on the Ochratoxin A (OTA) reduction in coffee beans (Coffea arabica)

O. Castellanos-Onorio a, O. Gonzalez-Rios a, B. Guyot b, Tachon A. Fontana b,*, J.P. Guiraud b, S. Schorr-Galindo b, N. Durand b, M. Suárez-Quiroz a

a Unidad de Investigacion y Desarrollo en Alimentos, Instituto Tecnologico de Veracruz, Mexico
b UMR Qualisud (CIRAD, Université Montpellier II), 34095 Montpellier Cedex 5, France

A R T I C L E   I N F O

Article history:
Received 23 July 2010
Received in revised form 14 January 2011
Accepted 25 January 2011

Keywords:
Roasted coffee
Green coffee
Coffee processing
Ochratoxin A

A B S T R A C T

The roasting of green coffee beans (Coffea arabica) artificially contaminated by Ochratoxin A (OTA) after inoculation with Aspergillus westerdijkiae was carried out with two different roasting techniques (Rotating Cylinder [RC] and Fluidized Bed [FB]). The green coffee beans were contaminated at two different toxin levels (L1 = 5.3 μg kg⁻¹ and L2 = 57.2 μg kg⁻¹). Different roasting points (light, medium, dark and very dark) were set according to the L’ color coordinate. The cylinder roasting conditions were 0, 3, 6, 9, 12, and 15 min at 230 °C and the fluidized bed roasting conditions were 0, 0.9, 1.7, 2.6, 3.5 and 4.3 min, at 230 °C. The roasted beans were compared for their physical properties (bean swell and weight loss) as well as for their residual OTA content. The results indicated that the OTA reduction was similar for the two contamination levels: 95.1% and 97.2% with the rotating cylinder and 81.3% and 79.2% with the fluidized bed at the maximal roasting time. The OTA degradation kinetics differed between the two processes. The complete degradation of OTA within the limit of this study (230 °C) was not observed but the rotating cylinder roasting was the most efficient technical process for the OTA reduction in a commercial dark roasted coffee (88%).

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Ochratoxin A (OTA) is a mycotoxin which contaminates a wide range of food commodities like cereals, coffee, nuts, dried fruits, wine, beer, grapes and grape juice. OTA has been shown to possess nephrotoxic, carcinogenic, immunosuppressive and teratogenic properties and was classified as carcinogenic for humans (group 2B) (IARC, 1993; Pfohl-Leszkowicz & Castegnaro, 1999). This secondary metabolite is produced by filamentous fungi such as Aspergillus carbonarius, Aspergillus niger, Aspergillus ochraceus, Aspergillus westerdijkiae and A. steynii as well as Penicillium verrucosum and Penicillium nordicum. In tropical zones, OTA is mainly produced in coffee beans by A. ochraceus and A. westerdijkiae (section Circumdati), which was recently dismembered from A. ochraceus, due to their important OTA production and occurrence (Bacha, Atoui, Mathieu, Liboz, & Lebrini, 2009; Frisvad, Frak, Houbraken, Kuipers, & Samson, 2004; Samson, Hong, & Frisvad, 2006; Taniwaki, Pitt, Teixera, & Imanaka, 2003).

The natural occurrence of OTA in green coffee beans has been reported since 1974 (Levi, Trenk, & Mohr, 1974). OTA in green coffee samples was usually found with concentrations ranging between 0.2 and 62 μg kg⁻¹ (Gopinandhan et al., 2008; Heilmann, Rehfelt, & Rotzoll, 1999; Romani, Sachetti, Graves Lopez, Pinnavaia, & Dalla Rosa, 2000). The presence of ochratoxin A in roasted coffee and in coffee brews was reported by Tsubouchi, Yamamoto, Hisada, Sakabe, and Udagawa (1987). Before this date, it was generally accepted that OTA was decomposed during roasting, nevertheless concentrations superior to the 20 μg kg⁻¹ have been reported in commercial roasted coffee (Mounjouenpou, Durand, Guyot, & Guiraud, 2007). Several reports concerning the roasting impact on OTA content in coffee beans have shown a large range of OTA reduction levels from 0–12% to 90–100% (Amezqueta, Gonzalez-Penas, Murillo-Arbizu, & Lopez de Cerain, 2009) Such variability could be related to the different analytical conditions or roasting process or heterogeneity in toxin distribution (Suárez-Quiroz et al., 2005; Van der Stegen, Essens, & van der Lijn, 2001). Some explanations for the OTA reduction during roasting were suggested: physical removal of OTA with husk, thermal degradation with possible involvement of moisture or isomerization into other diastereomers (Cramer, König, & Humpf, 2008; Van der Stegen et al., 2001).
Coffee is one of the most widely consumed beverages in the world and its contribution to the individual OTA dietary intake could be relatively high. The European Union legislation set up a maximum level for OTA in roasted coffee (5.0 μg kg⁻¹) and instant coffee (10.0 μg kg⁻¹) (European Commission, 2006) but level for OTA in green coffee is not yet restricted. However, in Europe, there are national limits for OTA in green coffee ranging from 5.0 μg kg⁻¹ in Finland to 20.0 μg kg⁻¹ in Greece (European Commission, 2002, 2006; FAO, 2006).

Conventional roasting is conducted in a rotating cylinder with internal paddles for mixing and tumbling beans, hot air blast assisted by centrifugal force carries the beans to the periphery; they then fall back to the center. Other coffee roasters currently used for large-scale roasting consist of fluidized bed and spouted bed roasters. Spouting bed technology increases the heat transfer but roasting can be inhomogeneous (Eggers & Pietsch, 2001). The degree of roasting is usually monitored through ground coffee color value from light to dark (Strezov & Evans, 2005). Some other important physical values like the bean type, size, mass and moisture are also useful parameters to appreciate roasting. The coffee beans are usually roasted for a period of 5 to 15 min at a maximum temperature of 250 °C. For example, end bean temperatures of 226 °C, 232 °C and 238 °C are recommended for Anglo tan, American light brown and European brown types respectively (Sivitz, 1991).

As it was shown above, data concerning the fate of OTA during roasting is disparate. Roasting experiments were often carried out far from usual practice or effective bean contamination. Results of studies on the kinetics of OTA degradation during roasting are also still scarce (Ferraz et al., 2010). Since kinetic studies have a real importance to prevent the occurrence of OTA in coffee brews, the purpose of this study was to improve the knowledge concerning the OTA destruction during coffee roasting. Different industrial roasting conditions used for commercial products were studied and compared.

2. Material and methods

2.1. Green coffee beans

Experiments were carried out using 3 lots of 6 kg of green coffee beans (Coffea arabica) from the Coatepec region, Veracruz, Mexico (2005–2006 harvest).

2.2. OTA artificial contamination of coffee beans

A toxigenic strain of A. westerdijkiae (before identified as A. ochraceus) Wilhelm MUCL 44640) isolated from coffee beans in Mexico was grown on potato dextrose agar (BD Bioxon) pH 3.5 at 25 °C for 5 days. Conidia were collected in 100 mL of a 0.1% Tween-80 solution by scraping of the surface of 3 culture plates. After counting on a Neubauer chamber, 300 mL of a 4.5 × 10⁷ conidia mL⁻¹ suspension were used to inoculate each lot of green coffee beans. The inoculated green coffee beans were placed into stainless steel trays after being disinfected with 4% sodium hypochlorite solution and 70% alcohol. The culture was incubated at 25 °C for 3 and 9 days.

2.3. Roasting procedures

Coffee drying: Before roasting, the coffee beans were dried gradually (50 °C for 2 days, 70 °C for 3 days and 90 °C) until 12% moisture content (Illy & Viani, 1995). Moisture content was assessed according to the ISO 11294 (1994) standard.

Rotating cylinder (Batch-type roaster): Samples (200 g) were roasted in a Probat-Werne type RE1 pilot roaster. Temperature was set at 230 °C, the operating conditions were 3, 6, 9, 12 and 15 min. After each time coffee beans were dried by air up reaching room temperature.

Fluidized bed roaster: Samples (200 g) were roasted in a Neuros Neotec pilot roaster. The air temperatures was set at 230 °C, the operating conditions were 0.9, 1.7, 2.6, 3.5 and 4.3 min. The different degrees of roasting – light, medium and dark – were obtained depending on the length of time spent by the coffee in both roasters. Bean swell, weight loss and color were used to assess the degree of roasting. Color was assessed with a colorimeter (ChromMeter CR-200 Minolta, Konica-Minolta France, Roissy, France) in reference to the CIELAB color space using D65 illuminant, which gave the dimensions L*, a*, b* used to characterize the color of the analyzed sample.

2.4. OTA quantification

In green coffee: Coffee samples were frozen at −80 °C then ground to pass a 0.5 mm sieve and analyzed for OTA content (Nakajima, Tsubouchi, Miyabe, & Ueno, 1997). The samples (10 g) were extracted for 30 min with 100 mL of methanol/3% sodium bicarbonate (50:50), the extracts (10 mL) were filtered and diluted with 30 mL of phosphate-buffered saline and applied to an immunoaffinity column (Ochrarep, R-Biopharm, Glasgow, UK). OTA was eluted with 3 mL HPLC grade methanol. The eluate was evaporated to dryness under a stream of nitrogen at 70 °C, and the residue was dissolved in 1 mL of HPLC mobile phase and then quantified by HPLC (Shimadzu LC-10ADVP, Japan, with fluorescence detector). The mobile phase consisted of distilled water/acetonitrile/glacial acetic acid (51:48:1). The injection volume was 100 μL and the flow rate was 1 mL min⁻¹. OTA was detected by absorption at 333 nm excitation and 460 nm emission at a retention time of 13.3–13.5 min. A standard curve of OTA was established from an ochratoxin A standard (1000 ng mL⁻¹; ref PD 226 R. Biopharm, Glasgow, UK) and the limit of detection was 0.05 ng mL⁻¹.

In roasted coffee: Ten grams of ground roasted coffee were extracted for 30 min with 100 mL of 3% methanol/bicarbonate solution (20/80) at 60 °C during 50 min; 5 mL of the filtered extract were diluted with 40 mL of PBS buffer and cleaned through an immunoaffinity column (Mounjouenpou et al., 2007). The OTA analysis of extract was then performed as for green coffee.

3. Results and discussion

3.1. OTA artificial contamination of green coffee beans

Two lots of 6 kg green coffee were inoculated by a suspension of spores of A. westerdijkiae reaching an initial concentration of 4.5 × 10⁶ conidia mL⁻¹. One third lot was used as control. After 3 and 9 days of incubation to 25 °C, green coffees containing respectively 5.3 and 57.2 μg kg⁻¹ of OTA were obtained. These levels of contamination were representative of values found in naturally contaminated green coffee samples. Micco, Grossi, Miraglia, and Brera (1989) reported that green coffee samples showed a significantly high contamination percentage (58%) of OTA ranging from 0.2 to 15 μg kg⁻¹. Heilmann et al. (1999) found a range of OTA-contaminated samples from 0.2 to 62 μg kg⁻¹ in a panel of 112 different coffee beans from 29 coffee-producing countries. Romani et al. (2000) showed that samples were positive for OTA with concentrations ranging from 0 to 48 μg kg⁻¹. In other reports, the OTA level in green coffee samples ranged from 1.3 to 31.5 μg kg⁻¹ (Napolitano, Fogliano, Tafuri, & Ritiendi, 2007; Pardo, Marin, Ramos, & Sanchis, 2004; Pérez de Obanos, González-Peñas, & López de Cerain, 2005). The discrepancies found on the effect of the roasting in the OTA content in coffee have been attributed to differences in the type and levels of contamination. In the present work the OTA contamination levels of coffee samples used were the most nearby to the average found in naturally contaminated samples.
3.2. Physical parameters of roasted beans

During the roasting the green coffee significantly alter its physical properties increasing the volume up to almost a double of the original size, loosing between 15 and 25% of the weight and continuously changing the color. The degree of roasting is usually monitored through visual means by the ground light reflectance and physical parameters (Strezov & Evans, 2005). The color expressed as L* value of one sample of commercial coffee roasting was used as reference. Variations in degree of roasting were light (L* value 31), medium (L* value 24), dark (L* value 19) and very dark (L* value 17–18). In this study two coffee samples (5.3 and 57.2 μg OTA kg⁻¹) were subjected to two different methods of roasting at the same temperature (230 °C). Table 1 gives the characteristics of the different roasts and roasting conditions. The light, medium and dark roasting degrees were obtained after 6, 9 and 12 min respectively in the rotating cylinder roaster and 0.9, 1.7 and 2.6 min in the fluidized bed roaster.

The very dark level was obtained after 15 and 3.5 min for the rotating cylinder and the fluidized, respectively. The bean swell reached then 35% in the rotating cylinder compared to only 25% in the fluidized bed. The weight loss (6%) was similar in the two roasters. These results were lower than those reported in the literature probably because of the operational conditions (Strezov & Evans, 2005).

Some considerable inconsistencies are found in the literature regarding the influence of roasting and subsequent operations on the OTA content of coffee (Viani, 2002). Levi et al. (1974) have shown that studies on roasting were carried out in experimental conditions from outside the common roasting practice to experiments in industrial scale equipment. Studies using artificially contaminated green coffee reported roasting conditions within the range of normal commercial practice (Micco et al., 1989).

3.3. OTA reduction according to the roasting conditions

Except for the very light roasting degree, there was no difference in the percent reduction in OTA content between the two contamination levels as well in the rotating cylinder as in the fluidized bed (Table 2).

Concerning the impact of the process on the OTA degradation, the rotating cylinder was most effective than the fluidized bed for a same degree of roasting: 96% OTA reduction against 75% for the very dark roasting degree (average between the two contamination levels) (Table 2). These results were consistent with those obtained with various coffee beans and roasting conditions (Ferraz et al., 2010; Van der Stegen et al., 2001).

The difference in OTA reduction between the two techniques depended on the heat diffusion in coffee beans. The observation of the transversal cross-sections showed that homogenous dark level was obtained for the whole beans with the rotating cylinder (12 min roasting time). In the fluidized bed (2.6 min roasting time), the thermal diffusion affected only the external bean layer. The internal OTA degradation of the coffee beans could not then occur.

An important standard deviation on OTA values was found at the very light degree. It was due to the important variability in moisture loss that is usually observed during this first roasting phase (Baggentoss, Poisson, Kaegi, Perren, & Eschert, 2008).

While only a low OTA reduction was observed during the light roasting time for the 2 processes, the most significant reduction took place between the medium and dark roasting degrees (Fig. 1). The OTA degradation during roasting can be attributed to both thermal destruction and chaff removal during the process (Heilmann et al., 1999). In the case of light roasting phase, the total amount of OTA that would have been removed with chaff remained

### Table 1
Roasting degree and physical properties of OTA-contaminated coffee samples for two methods at 230 °C.

<table>
<thead>
<tr>
<th>Roasting method</th>
<th>Roasting time (min)</th>
<th>Roasting degree</th>
<th>Color value ground coffee (L* value)</th>
<th>% Bean swell*</th>
<th>% Bean weight loss*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.3 μg kg⁻¹</td>
<td>57.2 μg kg⁻¹</td>
<td>Ref</td>
</tr>
<tr>
<td>Rotating cylinder</td>
<td>3</td>
<td>Very light</td>
<td>53.9 ± 9.6</td>
<td>40.2 ± 6.7</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Light</td>
<td>42.8 ± 7.6</td>
<td>32.0 ± 5.3</td>
<td>31.4 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Medium</td>
<td>23.2 ± 2.1</td>
<td>26.1 ± 2.0</td>
<td>24.6 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>Dark</td>
<td>19.4 ± 2.5</td>
<td>23.0 ± 2.9</td>
<td>19.0 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Very dark</td>
<td>16.7 ± 2.2</td>
<td>18.4 ± 1.5</td>
<td>30.4</td>
</tr>
<tr>
<td>Fluidized bed</td>
<td>0.9</td>
<td>Light</td>
<td>35.4 ± 0.1</td>
<td>35.3 ± 0.4</td>
<td>34.3 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>1.7</td>
<td>Medium</td>
<td>26.1 ± 1.0</td>
<td>27.5 ± 1.8</td>
<td>24.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>2.6</td>
<td>Dark</td>
<td>21.4 ± 1.7</td>
<td>23.7 ± 2.0</td>
<td>19.5 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>Very dark</td>
<td>19.8 ± 1.3</td>
<td>21.7 ± 1.4</td>
<td>24.4</td>
</tr>
<tr>
<td></td>
<td>4.3</td>
<td>Very dark+</td>
<td>20.0 ± 1.5</td>
<td>22.1 ± 1.6</td>
<td>29.6</td>
</tr>
</tbody>
</table>

* Results for 5 replicates.

### Table 2
Influence of two roasting methods on the OTA content in coffee beans at two contamination levels.

<table>
<thead>
<tr>
<th>Contamination level</th>
<th>Roasting time (min)</th>
<th>Roasting degree</th>
<th>OTA μg kg⁻¹</th>
<th>% OTA reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.3 μg kg⁻¹</td>
</tr>
<tr>
<td>Rotating cylinder</td>
<td>0</td>
<td>Very light</td>
<td>5.3 ± 0.1</td>
<td>57.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Light</td>
<td>5.3 ± 3.9</td>
<td>42.8 ± 25</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Medium</td>
<td>5.1 ± 1.5</td>
<td>46.4 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>Dark</td>
<td>1.7 ± 0.9</td>
<td>7.5 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Very dark</td>
<td>0.6 ± 0.1</td>
<td>1.6 ± 0.6</td>
</tr>
<tr>
<td>Fluidized bed</td>
<td>0</td>
<td>Light</td>
<td>5.3 ± 0.1</td>
<td>57.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>0.9</td>
<td>Medium</td>
<td>4.8 ± 0.6</td>
<td>33.0 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>2.6</td>
<td>Dark</td>
<td>1.9 ± 0.1</td>
<td>15.8 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>Very dark</td>
<td>1.4 ± 0.2</td>
<td>12.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>4.3</td>
<td>Very dark+</td>
<td>1.0 ± 0.2</td>
<td>11.9 ± 0.5</td>
</tr>
</tbody>
</table>

* Results for 5 replicates.
3.4. Kinetics of OTA degradation for two roasting procedures

The study of the OTA reduction during the roasting process showed that OTA degradation followed first order reaction kinetics. However, differences were observed using the two different techniques (Fig. 2).

The results obtained with the fluidized bed were in agreement with those obtained by Ferraz et al. (2010) using spouted bed roasting: the reduction rate constant was 0.38 min \(^{-1}\) \((R^2 = 0.925)\) at 230 °C in this study and was 0.43 min \(^{-1}\) at 240 °C in the spouted bed.

Concerning the rotating cylinder, the results showed two phases: a slow destruction rate from 0 to 6 min followed by a rapid destruction rate from 6 to 15 min roasting. Same results were found by Suárez-Quiroz et al. (2005) with a similar technique.

During the first degradation stage, the rate constant at 230 °C was 0.035 min \(^{-1}\) \((R^2 = 0.625)\) that was lower than those of Suárez-Quiroz et al. (2005) \((0.07\) min \(^{-1}\) at 200 °C and 0.18 min \(^{-1}\) at 250 °C). The low value of the R2 coefficient was due to the important standard deviation that was observed on OTA values during the first roasting phase related to the moisture loss variability. This first phase was probably the drying one \((T < 160 °C)\) while the second one was the roasting phase (Heyd, Broyart, Hernandez, Valdovinos-Tijerino, & Trystram, 2007).

For the second stage, the rate constant at 230 °C was 0.35 min \(^{-1}\) \((R^2 = 0.98)\) while Suárez-Quiroz et al. (2005) found 0.7 min \(^{-1}\) at 200 °C and 2.36 min \(^{-1}\) at 250 °C.

Considering the roasting process, the fluidized bed was insufficient for OTA degradation in order to obtain safe products when the level of green bean contamination was high and although the degree of roasting was very dark. Indeed, the OTA degradation rate was higher in the fluidized bed but the process time was too short to achieve a secure degradation level. The heat diffusion time was reduced and only the external layer of the coffee beans was reached by the thermal process and then OTA reduction was lower.

As well as for the moisture evaporation, the thermal effects occurring within the beans during roasting on the OTA degradation are strongly linked to the heating mode and the material properties of coffee. Some physical models which took into account the heat and mass transfers have been proposed to predict the temperatures of the bean center and surface (Basile & Kikic, 2009; Heyd et al., 2007). With some modifications these models can fit diverse roasting machines where the heat transfer takes place between air and bean and/or between air and metal and/or between metal and bean (Hernandez, Heyd, Irlés, Valdovinos, & Trystram, 2007). They were often established in order to control the organoleptic quality of coffee but could also be used to predict the OTA degradation.

4. Conclusion

In conclusion, the rotating cylinder was more effective for OTA degradation compared to the fluidized bed at the same roasting degree. The kinetics of OTA degradation were not influenced by the initial contamination level of green beans. However, as high the contamination level was as high the OTA residual was. Then, in some cases, the legal OTA level in roasted coffee in Europe \((5 \mu g kg \(^{-1}\))\) could not be reached.

Dark roasting, which is preferred in Europe, allowed a sufficient OTA reduction when the initial contamination level of the green beans was no more than 42 \(\mu g kg \(^{-1}\) \((12% \) residual OTA on average in the rotating cylinder). In the fluidized bed the initial OTA level should not exceed 15 \(\mu g kg \(^{-1}\) \((32% \) residual OTA on average). Light and medium roasting which are appreciated by American and Arabic consumers led to insufficient OTA reduction.

Depending on the process conditions and final product, the industrial processes could achieve a reduction of OTA level which could be very significant but which also greatly depends on the initial OTA contamination.

Trends to consume a less roasted coffee in order to preserve the antioxidant benefits of coffee that are decreasing with the thermal treatments disagree with OTA detoxification.

Thus, as shown in this work, industrial operators should not rely on roasting for OTA elimination and should avoid the processing of contaminated coffee beans.

Acknowledgements

The authors are grateful to the Consejo Nacional de Ciencia y Tecnología (CONACyT) Mexico for their financial support.