Food Control 21 (2010) 872–877

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Kinetics of ochratoxin A destruction during coffee roasting

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ARTICLE INFO

Article history:
Received 3 August 2009
Received in revised form 24 November 2009
Accepted 4 December 2009

Keywords:
Coffee
Ochratoxin A
Chlorogenic acids
Coffee roasting
Modeling

ABSTRACT

In the present study, *Coffea arabica* was artificially contaminated with spores of toxigenic *Aspergillus westerdijkiae*. The contaminated coffee was roasted in a vertical spouted bed roaster at four different temperatures (180 °C, 200 °C, 220 °C and 240 °C) and three different time periods (5, 8 and 12 min), in order to obtain more accurate results for the development of the kinetic model for ochratoxin A (OTA). Chlorogenic acids (CGA) content during coffee roasting was also evaluated to investigate the effect of the heat employed to destroy OTA in these health promoting compounds. Coffee treated with spouted bed roasting significantly reduced the OTA level from 8% to 98%. The spouted bed roasting proved to be a very efficient procedure for OTA reduction in coffee, and its reduction depended directly on the degree of roasting. OTA degradation during coffee roasting followed first order reaction kinetics. Using the apparent activation energy of OTA degradation and the temperature-dependent reaction rate, there was a compliance with the Arrhenius equation. This model was capable of predicting the thermal induced degradation of OTA and may become an important predicting tool in the coffee industry. The present study was also able to propose roasting conditions appropriate to destroy OTA and maintain most of the CGA at the same time.

1. Introduction

Coffee is one of the most popular and consumed food products in the world. Recently, epidemiological and clinical studies have been attributing beneficial health effects to this beverage, mainly due to its high content of phenolic compounds, which make coffee one of the highest contributors to antioxidant intake in western diets (Farah, 2009). In addition to phenolic compounds, coffee may also contain other potentially beneficial bioactive components such as the vitamin niacin and trigonelline, among others (Farah, 2009; Farah & Donangelo, 2006; Perrone, Donangelo, & Farah, 2008). Because of these bioactive components, coffee has been considered to be a potential functional food product (Farah, 2009). However, coffee may also contain undesirable compounds that may be intrinsic to the beans or produced during primary or industrial processing. One of the most relevant prejudicial compounds in coffee is ochratoxin A (OTA).

OTA consists of a polyketide-derived dihydroisocoumarin moiety linked through the 12-carboxy group to phenylalanine (Van der Merwe, Steyn, & Fourie, 1965). Its toxicity has been reviewed by the International Agency for Research on Cancer (IARC, 1993) which has classified OTA as a possible human carcinogen (group 2B). The kidneys are considered to be the main target organ for OTA effects, and nephrotoxic and carcinogenic properties have, therefore, been the major focus of the safety evaluation performed by scientific bodies (Krogh et al., 1988; Kuiper-Goodman, 1996).

In coffee, OTA is mainly produced by *Aspergillus westerdijkiae, Aspergillus steynii, Aspergillus ochraceus* and related species, and *Aspergillus carbonarius,* with a small number of isolates of *Aspergillus niger* (Frisvad, Thrane, Samson, & Pitt, 2006; Pardo, Marin, Ramos, & Sanchis, 2004; Taniwaki, Pitt, Teixeira, & Iamanaka, 2003; Urbano, Leitão, Vicentini, & Taniwaki, 2001). The contamination of coffee by fungi may take place when coffee fruits fall onto the ground and if acquire higher moisture content during drying and storage (Bucheli & Taniwaki, 2002; Pardo et al., 2004; Suárez-Quiroz et al., 2004; Taniwaki et al., 2003; Urbano et al., 2001). The presence of OTA in coffee was first reported by Levi, Trenk, and Mohr (1974) and, since then, systematic investigations on OTA incidence in green, roasted and instant coffee and brew have been extensively reported (Burdaspal & Legarda, 1998; Lombaert et al., 2002; Perez de Obanos, Gonzales-Penas, & Lopez de Cerain, 2005; Studer-Rohr, Dietrich, Schlatter, & Schlatter, 1995; Taniwaki, 2006; Taniwaki et al., 2003).

OTA is generally stable to the level of heat utilized in ordinary cooking. Boudra, Le Bars, and Le Bars (1995) showed that OTA is

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doi:10.1016/j.foodcont.2009.12.001

heat stable and only up to 20% of OTA in wheat was decomposed by dry heat at 100 °C for 160 min or 150 °C for 32 min. On the other hand, because of the high temperatures used for coffee roasting, a higher percentage of destruction is observed. However, contradictory results from different studies have been reported. Tsubouchi, Yamamoto, Hisada, Sakabe, and Udagawa (1987) reported that OTA in artificially contaminated green coffee beans was only slightly reduced (0–12%) by heat treatment at 200 °C for 10–20 min and that almost all the toxin was infused into the coffee decoction when the roasted samples were ground and extracted with boiling water. This is broadly in agreement with results of Studer-Rohr et al. (1995) but in contradiction with those of Micco, Grossi, Miraglia, and Brera (1989) and Blanc, Pittet, Munoz-Box, and Viani (1998) which showed that up to 80% of the OTA was destroyed during industrial roasting due to both thermal destruction and coffee chaff removal.

Even though coffee roasting may be an effective process for OTA destruction, the practice of severe roasting should not be used in an indiscriminate way, since other thermolabile compounds which are beneficial to human health, such as chlorogenic acids (CGA) may be destroyed. CGA are the main phenolic compounds in coffee (4–12% of green coffee composition), formed by the esterification of one or two molecules of certain *trans* cinnamic acids with (-)-quinic acid (Farah, DePaulis, Trugo, & Martin, 2005). The major classes of CGA in coffee, containing at least three important isomers, are caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA) and feruloylquinic acids (FQA). During roasting, CGA are lost as a consequence of the thermal breakage of carbon–carbon covalent bonds, resulting in molecule changes and degradation (Farah & Donangelo, 2006; Farah et al., 2005). Several beneficial health effects have been attributed to CGA and derivatives, mainly related to their potent antioxidant capacity. Other reported activities are hypoglycemic and antibacterial, among others (Almeida, Farah, Silva, Nunan, & Glória, 2006; Antonio et al., in press; Farah, 2009; Hemmerle et al., 1997; Herling et al., 1998; Shearer, Sellars, Farah, Graham, & Wasserman, 2007; Shearer et al., 2003).

Although kinetic studies are useful for food technologists to design specific processes with greater efficiency and avoid energy and product losses, few studies on the kinetics of OTA destruction in coffee have been carried out. In the present study, twelve roasting conditions were used to model OTA thermal degradation kinetics in coffee. Since the analysis of OTA in naturally contaminated green coffee beans is difficult due to the low contents found in these samples, we used artificially contaminated green coffee beans (inoculated with *A. westerdijkiae*), in order to obtain more accurate results for the development of the kinetic model. CGA content during coffee roasting was also evaluated to investigate the effect of the heat employed to destroy OTA in these health promoting compounds.

2. Material and methods

2.1. Coffee sample

Green *Coffea arabica* beans obtained by drying and de-husking of coffee fruits, were harvested in São Paulo State, Brazil and acquired directly from producers.

2.2. Selection and preparation of spore suspensions

A. westerdijkiae isolated from coffee grown in Brazil was inoculated into malt extract agar (MEA) and incubated at 25 °C for 5 days. The growing culture was transferred into a test tube containing 30 mL of 0.1% peptone water plus 0.1% tween 80, and the suspension was agitated in a vortex for 1 min. A spore concentration of 10^7 CFU/mL was obtained by dilution technique.

2.3. Inoculation of Aspergillus westerdijkiae spores into coffee beans

Sixteen 2 L Polyethylene Terephthalate (PET) flasks were surface decontaminated with 70% alcohol and dried under UV light for 15 min. Following this, 1 kg of green coffee beans was placed into each flask and 120 mL of the inoculum were added. Content was homogenized by hand shaking for 2 min. Additionally, 120 mL of peptone water 0.1% without fungal inoculum were added to one flask containing the same amount of green coffee beans to achieve similar water activity conditions and be used as a control. Flask tops were loosened and the flasks were incubated at 25 °C for 30 days. Flasks were shaken daily and opened for aeration inside an aseptic cabinet flow. After 30 days, all the *A. westerdijkiae* contaminated coffee and the non-inoculated control were homogenized and analyzed for OTA and CGA.

2.4. Coffee roasting

Coffee samples (300 g) were roasted in duplicate in a vertical spouted bed roaster (105 mm diam, 590 mm height, 20 mm screen, 45° angle) operating at 180, 200, 220 and 240 °C for 5, 8 and 12 min. The roaster was previously heated with hot air until the roasting temperature was achieved. After roasting, samples were immediately placed into a dry air cooler until reaching room temperature. Roasted coffee samples were ground in a Probat mill (Probat Emmerich Stawert Mühlenbau Typ K32/20, Germany) and sieved (20 mesh).

2.5. Roasting degree classification

Colorimetric values were obtained using an Agtron Model E10-CP spectrophotometer (Agtron, Reno, NV – USA) and compared with the color disks from the "Roasting Color Classification System" (Agtron) for determination of roasting degrees.

2.6. Ochratoxin A analysis

Ochratoxin A from green and roasted coffee was analyzed according to Vargas, Santos, and Pittet (2005). A 25 g aliquot of coffee samples was extracted with 200 mL of a mixture of methanol: 3% aqueous sodium bicarbonate (50:50). Extracts were filtered and diluted with phosphate buffered saline and applied to an immunoaffinity column (Vicam L.P., Watertown, MA) containing monoclonal antibody specific for OTA. After washing with distilled water, OTA was eluted with HPLC grade methanol and quantified by reverse-phase HPLC with fluorescence detection. A volume of 20 μL was injected through a SIL-10ADVP auto injector (Shimadzu Corporation, Japan). The mobile phase was methanol:acetronitrile:water:acetic acid [35:35:29:10, (v/v/v/v)], with an isocratic flow rate of 0.8 mL/min pumped by a SLC-10AVP (Shimadzu Corporation, Japan). The equipment used was a Shimadzu LC-10VP system (Shimadzu Corporation, Japan) with a fluorescence detector RF-10AXL (Shimadzu Corporation, Japan) set at 333 nm excitation and 477 nm emission. Chromatographic separation was achieved using a Supelcosil™ LC-18 column (5 μm, 250 × 4.6 mm, Supelco, Bellefonte, PA) equipped with an ODS Hypersil pre-column (5 μm, 25 × 4.6 mm) placed into a CTO-10ADVP column oven (Shimadzu Corporation, Japan) at 40 °C. The method to analyze OTA in green coffee was already validated. To recover determination, coffee samples were spiked with OTA standard (Sigma, USA) at three levels (4.8, 8.0 and 80.0 ng/g). The obtained OTA recoveries were 86.5%, 78% and 81%, respectively. The detection and quantification

limits of the method were 0.1 and 0.3 ng/g, respectively. OTA levels were expressed as ng/g of coffee on a wet basis.

2.7. Chlorogenic acids analysis

Green and roasted coffee samples were ground to pass through a 0.046 mm sieve and extracted in duplicate with boiling water, according to a modification of the method of Trugo and Macrae (1984), described in detail in Farah et al. (2005). Analyses by a HPLC gradient system using 10 mM citric acid solution and methanol as the mobile phase were performed as also described in Farah et al. (2005) and Farah, de Paulis, Moreira, Trugo, and Martin (2006). Chromatographic separation was achieved using a Rexchrom ODS-C18 column (5 μm, 250 × 4.6 mm, Regis Technologies, Morton Grove, IL) coupled with a guard column (Rexchrom, 5 μm, 10 × 3 mm). Detection was performed by a UV detector (Model SPD-10AVp Shimadzu, Japan), operating at 325 nm. The variation coefficient of the analytical method was lower than 5%. The detection limit for 5-CQA, the main representative of CGA class in coffee, was 1.7 μg/mL. CGA levels were expressed as g/100 g of coffee on a dry weight basis.

2.8. Water content

In order to express the amount of CGA per weight of dry matter, the water content of the freshly ground beans was determined according to AOAC (2000).

2.9. Kinetics of OTA degradation

The kinetic model used in this study was chosen because of the balance between its simplicity and the good performance of experimental data it provided. We assumed that the kinetic parameters followed the Arrhenius law. The dependence of the degradation rate constant on the temperature was quantified by the Arrhenius equation (Nisha, Singhal, & Pandit, 2005; Tarade, Singhal, Jayram, & Pandit, 2007; Özdemir & Devres, 2000).

3. Results and discussion

3.1. Production of ochratoxin A by A. westerdijkiae

The average content of ochratoxin A produced by *A. westerdijkiae* in the contaminated raw coffee sample after 30 days of incubation was 247 ng/g which is about 100 times the average values reported in the literature for naturally contaminated coffee beans (MAFF, 1996; Studer-Rohr et al., 1995; Taniwaki, 2006).

3.2. Roasting of the beans

Table 1 shows the colorimetric values and respective roasting degrees of coffee before and after being roasted in different roasting conditions. Color development was directly related to roasting time and temperature, and roasting degrees varied from very light to very dark.

Table 1
Roasting conditions, colorimetric values and roasting degree of the coffee samples used in this study.

Roasting conditions		Instrumental color[a] (Agtron scale)	Disc color #	Roasting degree
Temperature (°C)	Time (min)			
180	5	96.9 ± 0.0	95	Very light
	8	96.9 ± 0.0	95	Very light
	12	92.5 ± 0.1	95	Very light
200	5	96.9 ± 0.0	95	Very light
	8	85.8 ± 1.3	95	Very light
	12	66.3 ± 0.6	85	Light
220	5	88.6 ± 0.3	95	Very light
	8	55.9 ± 0.6	75	Moderately light
	12	44.7 ± 0.1	55	Medium
240	5	59.7 ± 0.4	75	Moderately light
	8	27.4 ± 0.2	35	Dark
	12	10.9 ± 0.7	25	Very dark

[a] Results are the average ± SD of three measurements.

Table 2
Ochratoxin A content and percent loss in green and roasted coffee samples used to compute the degradation model.

Roasting conditions		Ochratoxin A (ng/g)[a]	Ochratoxin A loss (%)	k (min^{-1})
Temperature (°C)	Time (min)			
	Green coffee	247 ± 20.1	–	–
180	5	227 ± 20.0	8.2	0.10
	8	143 ± 12.9	42.1	
	12	115 ± 5.6	53.6	
200	5	173 ± 31.7	29.9	0.11
	8	108 ± 9.1	56.1	
	12	80.6 ± 3.2	67.4	
220	5	132 ± 8.0	46.7	0.20
	8	58.9 ± 3.3	76.2	
	12	31.7 ± 8.0	87.2	
240	5	57.5 ± 9.5	76.7	0.43
	8	14.0 ± 2.9	94.3	
	12	2.8 ± 1.0	98.9	

[a] Results are the average ± SD of four replicates of analysis.

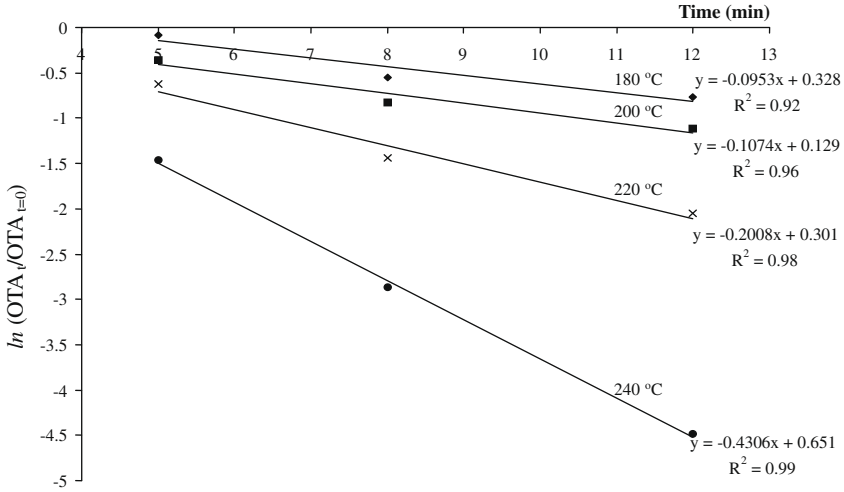

Fig. 1. First order plots of OTA degradation in coffee samples at 180, 200, 220 and 240 °C.

3.3. Ochratoxin A destruction

After roasting, coffee samples were analyzed for OTA content. Table 2 shows the contents of OTA in green and roasted coffee samples used to compute the degradation kinetic models. Percent reduction in OTA content due to roasting is also shown. After 12 min roasting, OTA content reduction ranged from 53% to 99% for samples roasted at 180 and 240 °C, respectively. Samples roasted at intermediate roasting temperatures showed intermediate OTA losses, as expected. Our results are in agreement with those obtained using naturally contaminated coffee beans (Blanc et al., 1998). On the other hand, the results of Tsubouchi et al. (1987) showed that OTA was slightly reduced (0–12%) by heat treatment at 200 °C for 10–20 min. This discrepancy may be mainly due to their using of a static oven in which the convection heat exchange is very low and a considerable part of the roasting time might have been used just for drying, with the beans remaining at the wet bulb temperature (estimated to be around 80–90 °C) for this experimental condition.

Evaluation of zero and first order equations, based on correlation coefficient, showed that the best fit ($R^2 > 0.88$) was obtained when OTA degradation reaction was considered to be of first order. Using linear regression analysis, the degradation rate equation data were analyzed as a function of temperature.

Fig. 1 shows the first order plots of OTA degradation in coffee samples at the tested temperatures. The half-life ($t_{½}$), time required for OTA to degrade to 50% of its original value, was calculated from the rate constant k, as '$\ln 2/k$'. The average rate constants for OTA degradation increased from 0.0687 to 0.3775 min^{-1} at 180 and 240 °C, respectively. A corresponding decrease in half-life, from 10.09 to 1.84 min, respectively, was observed.

The linear nature of the obtained plot ($R^2 > 0.90$) indicates that thermal destruction of total OTA conforms to the Arrhenius equation. Apparent activation energies E_a (kJ mol^{-1}) were calculated as a product of the gas constant R (8.3145 J mol^{-1} K^{-1}) and the slope of the graph obtained by plotting '$\ln k$' vs. '$1/T$'. The apparent activation energy for OTA thermal degradation was found to be 49.2 kJ mol^{-1}. The frequency factor A_0 (min^{-1}) was calculated as the exponential of the intercept of the Arrhenius plot at $1/T = 0$ and found to be 1.26×10^5.

The degradation of OTA during coffee roasting was used implementing the calculated rate constants for OTA degradation, k. A strong correlation ($R^2 = 0.96$) between experimental and calculated values was obtained (Fig. 2). An average relative error of 9.1% was found between experimental and calculated values. This relative error is in agreement with those reported by other modeling studies in the literature (Nisha et al., 2005; Tarade et al., 2007).

3.4. Chlorogenic acids destruction

Because CGA are biologically relevant thermolabile compounds in coffee, their destruction during coffee roasting was also evaluated. The contents of total CGA and of CQA, FQA and diCQA classes, as well as CGA percent loss in the coffee samples used in this study are presented in Table 3. CGA content in green coffee was 5.98 g/100 g, which is in agreement with numerous studies in the literature (Clifford, 1999; Farah & Donangelo, 2006; Farah et al., 2005; Perrone et al., 2008; Trugo & Macrae, 1984). As expected, CGA content reduced during roasting, with the exception of the sample roasted at 180 °C for 5 min. After 12 min roasting, CGA loss ranged from ~17% to 99% for samples roasted at 180 and 240 °C, respectively. Fig. 3 presents the percent loss of both OTA and CGA contents due to coffee roasting. It is possible to observe that OTA percent loss was more pronounced that CGA percent loss when coffee was roasted at 180, 200 and 220 °C. When the roasting temperature was set to

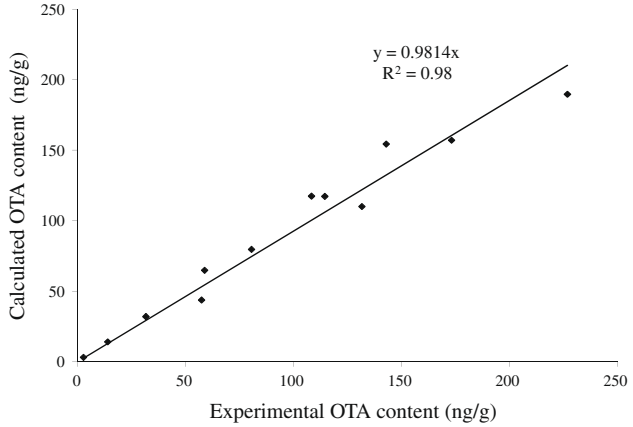

Fig. 2. Comparison of experimental and calculated OTA content (ng/g) in roasted coffee samples using a kinetic model based on a first order reaction rate and the Arrhenius law.

Table 3
Chlorogenic acids contents and percent loss in green and roasted coffee samples.[a]

Roasting conditions		Total CGA (g/100 g)[b]	CQA (g/100 g)[b]	FQA (g/100 g)[b]	diCQA (g/100 g)[b]	Total CGA loss (%)
Temperature (°C)	Time (min)					
	Green	5.98 ± 0.03	4.84 ± 0.02	0.15 ± 0.00	0.90 ± 0.01	–
180	5	6.17 ± 0.05	5.13 ± 0.03	0.17 ± 0.00	0.85 ± 0.02	3.0
	8	5.73 ± 0.29	4.87 ± 0.22	0.15 ± 0.01	0.70 ± 0.05	4.2
	12	4.99 ± 0.13	4.25 ± 0.12	0.17 ± 0.01	0.56 ± 0.01	16.6
200	5	5.69 ± 0.58	4.78 ± 0.41	0.20 ± 0.11	0.71 ± 0.05	4.9
	8	4.26 ± 0.42	3.67 ± 0.30	0.17 ± 0.05	0.42 ± 0.08	28.7
	12	3.23 ± 0.08	2.80 ± 0.10	0.14 ± 0.03	0.28 ± 0.01	45.9
220	5	4.52 ± 0.41	3.87 ± 0.30	0.19 ± 0.07	0.46 ± 0.04	24.4
	8	2.14 ± 0.12	1.90 ± 0.12	0.09 ± 0.01	0.15 ± 0.02	64.2
	12	1.20 ± 0.17	1.06 ± 0.11	0.09 ± 0.04	0.06 ± 0.01	79.9
240	5	2.50 ± 0.16	2.21 ± 0.14	0.11 ± 0.01	0.18 ± 0.01	58.2
	8	0.35 ± 0.02	0.29 ± 0.01	0.05 ± 0.01	0.01 ± 0.00	94.2
	12	0.05 ± 0.01	0.04 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	99.1

[a] CGA = chlorogenic acids; CQA = caffeoylquinic acids; FQA = feruloylquinic acids; diCQA = dicaffeoyl-quinic acids.
[b] Results are the average ± SD of two replicates of analysis.

240 °C, however, the percent losses of both compounds are very similar, especially after longer periods of roasting (8 and 12 min). To better compare the roasting conditions in terms of efficiency to destroy OTA and retain CGA, we calculated the ratio between CGA and OTA percent losses. Some restrictions were made, since only high efficiencies of OTA destruction (>75%) were used. Two samples showed OTA percent losses nearly to 80%, namely those roasted at 220 °C for 8 min and at 240 °C for 5 min. These samples were both classified in terms of roasting degree as moderately light (disc #75) and their calculated ratios (CGA/OTA) were 0.84 and 0.76, respectively. The samples roasted at 220 °C for 12 min and 240 °C for 8 and 12 min. showed roasting degrees as medium (disc #55), dark (disc #35) and very dark (disc #25), and their calculated ratios (CGA/OTA) were 0.92, 1.00 and 1.00, respectively. In this way, lower roasting temperatures for longer periods of time would favor OTA destruction and CGA retention. Nevertheless, it is clear from our results that in order to destroy most of the OTA present in the coffee, CGA content was significantly affected. It should be noted, however, that the nature of the cell wall of coffee beans probably changed during incubation, which could have facilitated the destruction of CGA and also, to a lower extent, OTA.

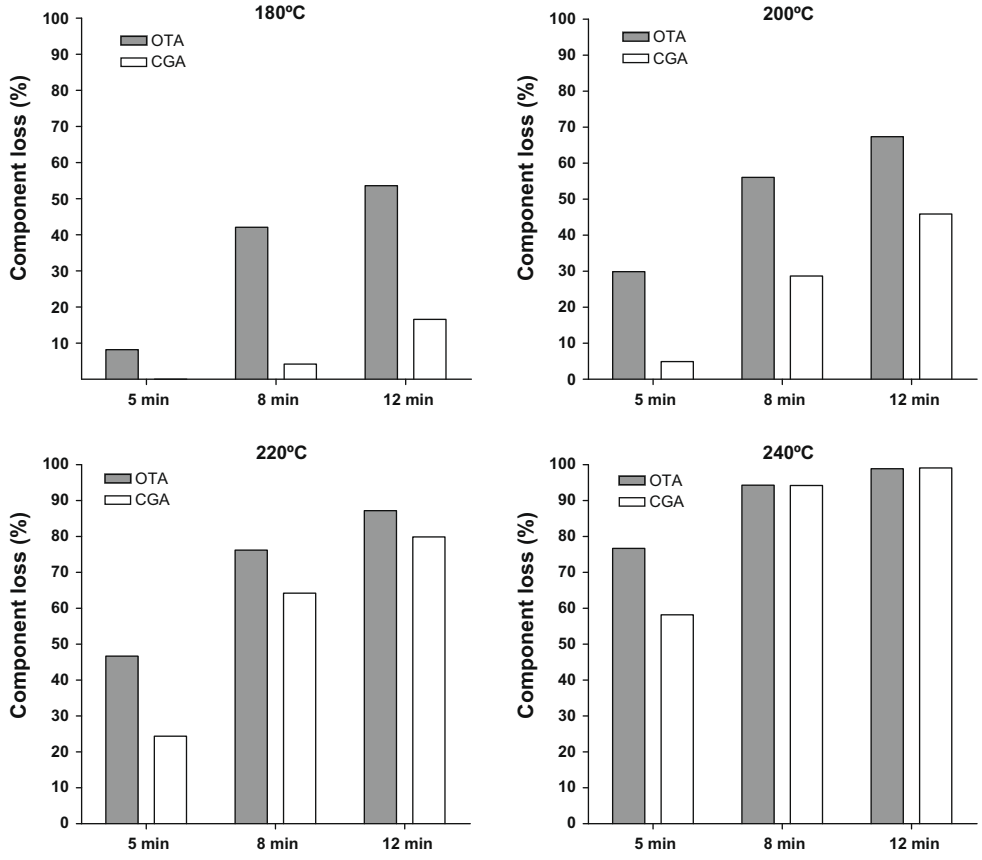

Fig. 3. Percent loss in OTA and CGA contents when green coffee was for roasted at 180, 200, 220 and 240 °C for 5, 8 and 12 min.

In conclusion, in the present study, we showed that spouted bed roasting is a very efficient procedure for OTA reduction in coffee, and its reduction depends directly on the degree of roasting. OTA degradation during coffee roasting followed first order reaction kinetics. Using the apparent activation energy of OTA degradation and the temperature-dependent reaction rate, a compliance with the Arrhenius equation was shown. This model was capable of predicting the thermal induced degradation of OTA. The proposed model may become an important predicting tool in the coffee industry. However, it should be kept in mind that using contaminated beans will greatly affect the quality of the beverage and that severe roasting of coffee beans to destroy OTA will also directly affect the levels of CGA, which are beneficial compounds to human health. Therefore, even though roasting may destroy OTA, the use of highly contaminated beans in coffee blends is not a recommended practice.

Acknowledgements

This work was funded by the Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café – EMBRAPA. The Support from CNPq and FAPERJ (Brazil) are gratefully acknowledged.

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