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Influence of Roasting Levels on Ochratoxin A Content in Coffee

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Because of inconsistent and contradictory results from investigations concerning the influence of roasting process on the ochratoxin A content in coffee beans, a study was undertaken to assess the elimination of ochratoxin A during the roasting process. Four different green coffee samples, naturally contaminated with ochratoxin A, were submitted to different roasting conditions (light, medium, and dark) and analyzed for roasting parameters (weight loss, color change, density, and moisture content) and ochratoxin A content. The ochratoxin A content of green coffee was reduced by the roasting process; in particular, consistently high percentages of ochratoxin A reduction were found in the highest contaminated samples. This reduction was influenced by the severity of the thermal process and was generally related to the initial ochratoxin A content. Samples obtained with roasting parameters suitable for a typical Italian espresso coffee brew showed reductions of >90% in the ochratoxin A content, in both high and low contaminated samples. Moreover, the presence of off-flavors and visual defects was not found to be directly related to the ochratoxin A content in the green coffee samples.

KEYWORDS: Ochratoxin A; coffee; roasting; reduction

INTRODUCTION

Ochratoxin A (OTA), which is a potent nephrotoxin and nephrocarcinogenic mycotoxin, can occur in a wide range of unprocessed and processed food including coffee (I). Within Europe, coffee accounts for \sim 7% of total ochratoxin A intake for humans (2).

The occurrence of the contaminant ochratoxin A in green and roasted coffee has been described widely in the literature (3-14). In particular, Romani et al. (15) showed that 106 of 162 samples of green coffee beans from various countries were positive for OTA, with concentrations of up to 48 ppb.

Results of brewing experiments (12, 16, 17) demonstrated that, with the predominantly used brewing methods, almost all OTA present in roasted coffee passes into the brew.

Because of the relatively high frequency of ochratoxin A contamination in green coffee and the difficulty of controlling the sanitary quality of this commodity, the roasting process, as a necessary process step to obtain the brew, appears to be an efficient way to reduce the OTA level and minimize the risk of its intake from coffee consumption. Moreover, the cleaning of green coffee (18) also contributes to the reduction of OTA.

To our knowledge, investigations concerning the effect of the roasting process on the ochratoxin A content in coffee beans have produced inconsistent and contradictory results (19), and it has not been established if and how much the OTA content could be reduced during the roasting process. The literature data (3, 6, 8, 17, 18, 20–22) show that the percentage of OTA destroyed by the thermal treatment of coffee ranges from 0 to 100%. Factors that may affect the results are heterogeneity of natural coffee bean contamination, natural contamination versus spiking, initial ochratoxin A levels, analytical method performance, and roasting conditions (23). The aim of this work was to study the effects of different roasting conditions such as time and temperature on the OTA content of naturally contaminated green coffees.

MATERIALS AND METHODS

Raw Material. Experiments were carried out on four green coffee bean samples, provided by a coffee roasting company. The sampling was conducted by the provider, according to an ISO method (24), although some authors (25) have suggested more a accurate sampling procedure, but one that is not commonly performed by company. Ten aliquots of ~ 300 g each were taken at different sites for each lot (100 bags per lot, 60 kg per bag). The samples used in these trials were from the Ivory Coast (sample A) and Zaire (different lots: samples B–D). These lots had been previously identified as containing a relatively high amount of ochratoxin A. Each sample (3 kg in size) was divided into subsamples for roasting; green samples were withdrawn from each subsample for analysis.

Visual Examination of Green Coffee Beans. Before roasting, the coffee samples were submitted to a visual and olfactory inspection by individuals experienced with the product, to evaluate the presence of evident defects such as broken, immature, fermented, and off-flavored (musty) beans. The presence of foreign matter such as cherry and parchment fragments (26) was also evaluated.

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Roasting. All coffee samples were roasted in a hot air circulating laboratory roaster of 1 kg per batch capacity, model 500 (STA, Crespellano, BO, Italy).

The roasting air temperature was 450 $^{\circ}$ C; each sample was submitted to different roasting times to obtain subsamples with different degrees of roasting: light for 6 min, medium for 7 min, and dark for 9 min. The temperatures reached within coffee sample beds before cooling were, respectively, 175, 185, and 204 $^{\circ}$ C.

Roasting levels were assessed on the basis of roasting parameters evaluated as given below. All of the chaff eliminated during roasting was collected

Grinding. Each sample was finely ground at room temperature using an Officine Vittoria grinder (Bologna, Italy) equipped with conical cutters to an average particle size of $\sim \! 0.4$ mm. The resulting powder was well homogenized by manual stirring to eliminate any OTA contamination differences.

Roasting Parameters. To evaluate the uniformity of the roasting process, the following parameters were evaluated on the green and roasted coffee samples:

Weight loss (percent) was determined by weighing coffee batches before and after roasting.

Color change was measured by using a tristimulus colorimeter (Chromameter-2 Reflectance, Minolta), equipped with a CR 100 measuring head. The standard C.I.E. conditions, with illuminant C (6774K) were used. The instrument was calibrated on a white tile ($L^* = 95.3$, $a^* = -1.0$, $b^* = 0.8$). Color is expressed in L^* (lightness). The samples were prepared by placing ground coffee or whole beans on suitable supports. Mean values and standard deviations were obtained from five measurements for each sample.

Density (grams per liter) was determined by using a suitable picnometer according to the methodology described by Lerici et al. (27).

Moisture content was determined by mass loss of ground coffee samples, after heating in an oven according to AOAC method 968.11 (28). Measurements were made in triplicate.

Ochratoxin A Analyses. OTA analyses were performed on all green and roasted samples using the high-performance liquid chromatography (HPLC) method of Pittet et al. (10), which may be summarized as follows. Finely ground coffee beans (25 g) were blended with 500 mL of methanol/3% aqueous sodium hydrogen carbonate (50:50 v/v) and filtered through a Whatman GF/B glass microfiber filter under reduced pressure. A 4 mL aliquot of filtrate was diluted to 100 mL with phosphate-buffered saline (PBS), and the whole diluted extract was applied to a Vicam Ochra Test immunoaffinity column (Vicam Inc., Watertown, MA), at a flow rate of 2-3 mL/min. After the column was washed with 10 mL of distilled water, OTA was eluted with 4 mL of methanol. The eluate was then evaporated to dryness under a stream of nitrogen at 40 °C, and the residue was redissolved in 150 µL of 45% acetonitrile-55% 4 mM sodium acetate/acetic acid (19:1 v/v). OTA was then identified and quantified by an HPLC system equipped with a fluorescence detector operated at an excitation wavelength of 333 nm and an emission wavelength of 460 nm.

The liquid chromatograph utilized was a Jasco system model LC-1500 (Carpi, MO, Italy) connected with a Jasco fluorescence detector model FP 1520.

Quantification was carried out by comparison to an external standard curve using an ochratoxin A standard (Sigma, purity > 98%). The OTA detection limit was <0.1 μ g/kg. Mean recoveries from spiked samples (n=4) at the 20 μ g/kg OTA level were 96.87% (RSD = 4.9%) for the green coffee and 109.5% (RSD = 2.1%) for the roasted coffee. The determined OTA contaminations were not corrected for recoveries.

Ochratoxin A analyses were made in triplicate for each sample. Data are reported as the average value of three determinations on separate extracts, obtained from three powder quotas for each subsample.

Statistical Analysis. Analysis of variance was performed on data by adopting the Tukey test, to test the statistical significance of the differences between means at a $p \le 0.05$ confidence level.

The data were processed using the Statistica for Windows (Statsoft, Tulsa, OK) package.

Table 1. Overall Results of Coffee Sample Roasting Parameters as a Function of Roasting Level

roasting				roasting level ^a		
parameter	sample	green	light	medium	dark	
moisture ^b (%)	A	9.39	2.13 a	0.95 b	0.49 c	
	B	10.15	2.29 a	0.86 b	0.47 c	
	C	9.02	2.29 a	0.71 b	0.41 c	
	D	10.07	2.18 a	0.91 b	0.47 c	
L*b	A	57.20	48.36 a	41.40 b	30.20 c	
	B	52.32	48.12 a	40.02 b	31.22 c	
	C	51.52	47.20 a	39.16 b	29.82 c	
	D	52.98	48.04 a	40.37 b	30.74 c	
wt loss ^c (%)	A B C D		13.09 e 13.79 e 13.11 e 13.64 e	16.06 d 17.26 d 19.31 c 16.89 d	21.64 a 21.09 a 20.69 b 20.43 b	
density ^c (g/L)	A	1.14	0.72 b	0.69 c	0.57 e	
	B	1.12	0.76 a	0.70 bc	0.58 e	
	C	1.04	0.74 a	0.64 d	0.55 e	
	D	1.14	0.74 a	0.69 c	0.56 e	
L*c	A	41.18	37.64 a	33.80 b	25.72 c	
	B	43.08	36.32 a	32.50 b	25.72 c	
	C	42.74	36.70 a	29.32 b	25.62 c	
	D	40.10	37.48 a	32.84 b	26.30 c	

 $[^]a$ Mean values and ANOVA results. Data marked with the same letter are not significantly different at a p < 0.05 level. b Ground coffee samples. c Coffee bean samples.

RESULTS AND DISCUSSION

Visual Examination of Green Coffee Beans. Coffee samples A and C showed strong off-flavors (musty and stale). Samples B and C showed the highest amount of dark (probably fermented or immature or infected) beans. Sample B showed the highest amount of broken and decayed beans. Only sample D did not exhibit any of these defects.

Roasting Parameters. As is well recognized, the most important physical changes that occur in coffee beans during the roasting process are weight loss, color change, and density reduction (27, 29-31). Each sample was submitted to different roasting conditions and analyzed for the previously mentioned parameters together with the moisture content; the data are reported in Table 1. On the basis of these data, the coffee samples roasted at the medium level presented the characteristics suitable for preparing an American-style "drip" coffee brew, and coffee samples roasted at the dark level were suitable for preparing an Italian-style espresso coffee brew. On the basis of ANOVA analysis, the roasting parameters, L^* , and moisture of the four different coffee samples processed at each roasting level (light, medium, and dark) did not show significant differences at the p < 0.05 level within the same roasting group. This result indicates that the roasting process was uniformly conducted. The slight differences observed in weight loss and density parameters were probably due to intrinsic differences existing between the green coffee samples. The values obtained for these parameters between different samples are within the typical ranges for the defined roasted degrees (32).

Influence of Coffee Roasting on Ochratoxin A Content. The results of OTA analyses carried out on green coffee and on samples roasted at different levels are reported in **Table 2**. As shown by the standard deviation values, OTA contamination was less homogeneous in the most contaminated green coffee samples (B and D, respectively). Moreover, it appeared that the presence of off-flavors and visual defects was not directly related to the OTA levels found in the green coffee samples, as observed

Table 2. Ochratoxin A Contents in Green and Different Roasted Coffee Samples

sample	roasting level	OTA content ^a (µg/kg)
А	green light medium dark	1.99 ± 0.36 a 2.01 ± 0.16 a 1.61 ± 0.38 a 0.18 ± 0.21 b
В	green light medium dark	29.30 ± 5.20 a 6.47 ± 1.54 b 2.78 ± 0.70 c 0.95 ± 0.17 d
С	green light medium dark	2.82 ± 0.18 a 0.71 ± 0.05 b 0.60 ± 0.24 bc 0.16 ± 0.17 c
D	green light medium dark	$\begin{array}{c} 9.64 \pm 1.47 \ a \\ 3.46 \pm 1.19 \ b \\ 1.09 \pm 0.33 \ c \\ 0.47 \pm 0.14 \ d \end{array}$

 $[^]a$ Mean values \pm standard deviation and ANOVA results. Data marked with the same letter are not significantly different at the p < 0.05 level.

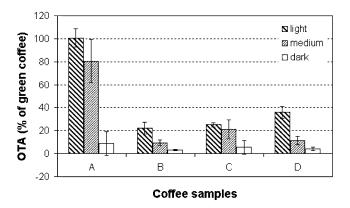


Figure 1. Ochratoxin A levels of roasted coffee samples reported as percent of ochratoxin A content initially in the respective green coffee.

by Leoni et al. (33), who found no correlation between OTA contamination and defective beans. Other studies (34, 35) have shown that the OTA content of green coffee appears to be linked to the amount of defects such as broken beans, infested beans, black beans, and husks. The latter defect seems to be the greatest source of OTA contamination found in green coffee.

Ochratoxin A content of green coffee was generally reduced by the roasting process. In absolute terms, the most significant OTA reduction was observed in the highest contaminated samples (B and D). In samples B and D, statistically significant reduction of OTA can be observed after each roasting level, whereas sample A showed significant OTA reduction only when processed at the dark roasting level. Sample C showed a gradual but not always significant reduction of OTA as a function of the severity of the thermal process.

For a better understanding of the data, the ochratoxin A level of roasted coffee samples is reported as a percentage of the OTA content initially in the respective green coffee (**Figure 1**). In samples B and C >70% OTA reduction was observed after the light roasting process, whereas sample A showed a completely different behavior. In all cases, the dark-roasted samples contained on average <10% of the OTA originally present in the green beans. This implies than even in the most contaminated sample the final OTA level after dark roasting was very low (<1 μ g/kg).

Table 3. Overall Ochratoxin A Reduction Due to Roasting Process and Chaff Removing

material	quantity (kg)	mean OTA concn (μg/kg)	total amount of OTA (μ g)	residual OTA (%)
green coffee roasted coffee	12.00 9.93	10.94 1.71	131.28 16.98	100.00 12.93
chaff 0.6%	0.07	38.00	2.74	2.08

The OTA reduction that took place during the roasting process can be attributed to both thermal degradation and chaff removal (13, 17, 19, 22). As it was not possible to accurately determine the proportion of chaff removed during roasting and to collect for each roasted sample an amount of chaff sufficient to perform an analysis, an overall chaff sample, ~0.6% of total green coffee weight, was recovered after the roasting of all samples and analyzed for OTA. The level of OTA in the chaff sample was 38 μ g/kg; thus, a residual percentage of OTA in the chaff was calculated on the basis of the recovered chaff amount (0.6%) according to the method of Blanc et al. (18), taking into account an overall initial green bean sample with an average value of contamination. In the same way, roasted coffee quantity and contamination level have been calculated as average values of different roasted coffee samples. Data are reported in Table 3. In comparison with the residual OTA percentage due to roasting process (12.93% on initial averaged contamination) the total amount of OTA that has been removed with chaff is small (2.08%). These results are consistent with those of Blanc et al. (18) even if the chaff contamination we found was \sim 4 times lower than that reported by them, probably due to the different roasting conditions.

In conclusion, the OTA reduction during roasting appears to be attributed mainly to a thermal destruction. This reduction was also influenced by the severity of the thermal process and was somewhat related to initial OTA content. The processing conditions adopted to obtain samples suitable for a typical espresso coffee brew were shown to eliminate >90% the OTA content in both high and low contaminated samples.

SAFETY

Ochratoxin A is a potent nephrotoxin and liver toxin and has been reported to have immunosuppressant properties. It is classified as possibly carcinogenic to humans (group 2B) by the International Agency for Research of Cancer (IARC). Gloves and safety glasses should be worn at all times, and all standard and sample preparation stages should be carried out in a fume hood.

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