Survey of the Presence in Green Coffee of Substances Associated with Important Off-flavours, and their Correlation with Ochratoxin A Contamination

G. BORTOLI, M. FABIAN

Demus S.p.A., via Caboto 31, 34147 Trieste, Italy. www.demus.it - E-mail: info@demus.it

SUMMARY

In this work an analytical method is developed for the determination of molecules responsible of important off-flavours related to fungal metabolism, such as 2,4,6-trichloroanisole and geosmin. The analysis was carried out by steam distillation of regular and decaffeinated green coffee, followed by qualitative and quantitative determination with GC-MS. In the meantime, on the same samples the concentration of ochratoxin A was determined by clean-up on immuno-affinity column followed by HPLC analysis.

The comparison of the results obtained evidences some correlation between the presence of molecules responsible of off-flavours and ochratoxin A in regular green coffee samples, which happens most evidently in coffees belonging to the Robusta species grown in some African Countries.

The analyses after decaffeination confirm the good extractive power for 2,4,6-trichloroanisole and geosmin of the solvent used in the process, methylene chloride. The final concentration of these analytes falls below the detection limit for all the samples, implying a useful effect of this extractive process over the off-flavours considered.

RÉSUMÉ

Dans ce travail on a mis au point une méthode d’analyse pour la détermination de molécules responsables d’importants arômes négatifs associées à des métabolismes mycosiques, tels que 2,4,6-trichloroanisole et géosmine. On a effectué cette analyse par distillation sous courant de vapeur sur des échantillons de café vert regular et décaféiné, suivie d’une détermination qualitative et quantitative par GC-MS. En même temps, sur les mêmes échantillons, on a déterminé la teneur en ochratoxine A par clarification sur colonne d’immunoaffinitè et par successive analyse HPLC.

A la lumière des resultats obtenus, on a mis en évidence une correlation entre la présence de molécules responsables d’arômes negatifs et la contamination par ochratoxine A dans les échantillons de café vert regular, quasi exclusivement dans les Robusta provenant des pays africains.

Les analyses effectuées après la décaféination confirment l'excellente capacité d’extraction du solvant utilisé dans le procédé, le chlorure de méthylène, par rapport à 2,4,6 trichloroanisole et géosmine. La concentration finale de ces analytes va au dessous de la limite de détection pour tous les échantillons, ce qui amène un effet positif du procédé d’extraction sur les d’arômes négatifs en question.

INTRODUCTION

Coffee flavour is one of the main reasons that attract consumers' attention and preference to this beverage. While, generally speaking, the definition of coffee quality is a complex issue, there is vast agreement that the presence of some off-flavours negatively affects the cup's appeal (Illy and Viani, 1995). Moreover, several off-flavouring taints have been ascribed – at least tentatively – to biological spoilage by micro-organisms such as bacteria or moulds (Liardon et al., 1989).

Important off-flavours in green coffee, subject of several studies, have been related to the presence of molecules such as 2,4,6-trichloroanisole (TCA) (Spadone et al., 1990), geosmin (GEO) (Gravesen et al., 1994; Johnsen and Kuan, 1987) and 2-methylisoborneol (Johnsen and Kuan, 1987; Vitzthum et al., 1990).

These substances, deriving from fungal metabolism, have an olfactory perception threshold extremely low, at the level of ppt (ng/kg) in coffee beverages, so that concentrations of ppb (μg/kg) have to be searched for in green coffee. This makes the realisation of an analytical method for their determination difficult, along with the problem that TCA and GEO are extracted together with the lipidic fraction, which covers the signal of the analytes of our interest (Tentindo, 1999).

The purpose of this work is to research the presence of TCA and GEO in green coffee, to verify if there are any differences between Arabica and Robusta samples, and to highlight the influence of the geographical area of production. In parallel, the contamination by another obnoxious fungal metabolyte, ochratoxin A (OTA), is investigated, with the purpose to find possible correlation between the presence of these off flavours and OTA.

Furthermore, the influence of solvent decaffeination process on off-flavours is investigated.

EXPERIMENTAL

The 75 lots of green coffee for the analysis, coming from 19 different countries, were selected by Demus S.p.A., Trieste and taken according to the ISO sampling method (1982). The analytical method to determine TCA and GEO was developed by G. Tentindo at the Department of Food Sciences of the University of Udine (Tentindo, 1999).

The same samples were analysed for Ochratoxin A (OTA) contamination. The study encompassed also 9 samples of decaffeinated coffee, to evaluate the effect of the treatment with methylene chloride on the molecules of our interest.

Determination of TCA and GEO

Each sample was completely ground, put in a distillation flask together with 2,3,6-trichloroanisole as internal standard and methanol, and submitted to steam distillation; then a three-step extraction with a solution of pentane/methylene chloride was performed on the distillate, and the heavy fraction dried with sodium sulphate. Then the sample was stored at –20°C until the GC-MS analysis.

For qualitative and quantitative determination was utilised a GC Varian 3400, coupled with a mass spectrometer Saturn ion trap (IDTMS). The capillary column was a DB5 (J&W) in fused silica, 30 m, i.d. 0.25 mm, with a film diameter of 0.25 μm. As transport gas was utilised helium and the injection was realised in splitless.

The detection limits were of 0.03 ppb for TCA and 0.2 ppb for GEO.

Determination of OTA

The analysis was effectuated on the same samples as above. Each sample was completely ground, homogenised and submitted to extraction, immuno-affinity clean-up and HPLC-spectrofluorimetry analysis as described by Studer-Rohr et al. (1995).

RESULTS

Correlation TCA - GEO – OTA

Table 1 shows the results of our analyses, reporting the average concentrations of TCA and GEO, together with the OTA average data, for 75 samples divided by geographical zone of production. The data highlight a higher OTA contamination in West and East African coffees.

Table 1.

SAMPLE	TCA		GEO		OTA	
	ppb	St.d.	ppb	St.d.	ppb	St.d.
West Africa 1 (R)	2,4	1,1	n.d.		1,1	1,6
West Africa 2 (R)	3,0	2,6	0,8	2,1	3,8	8,4
West Africa 3 (R)	4,8	2,8	0,8	1,1	3,0	2,1
West Africa 4 (R)	2,9	2,1	n.d.		2,5	4,8
East Africa 1 (A)	1,0	1,1	n.d.		2,2	4,2
East Africa 2 (R)	2,5	2,2	n.d.		2,7	5,3
East Africa 3 (R)	0,4	0,4	n.d.		0,6	0,4
Asia 2 (A)	n.d.		n.d.		0,1	0,3
Asia 1 (R)	0,6	0,9	n.d.		0,2	0,4
Asia 3 (R)	0,1	0,2	n.d.		0,2	0,3
Asia 4 (R)	0,1	0,2	n.d.		1,3	1,8
Central America 1 (A)	n.d.		n.d.		0.1	0.2
Central America 2 (A)	n.d.		n.d.		0,1	0,1
Central America 3 (A)	0,1	0,2	n.d.		0,4	0,8
Central America 4 (A)	0,8	0,1	n.d.			
Central America 5 (A)	0,1	0,2	0,1	0,2	0,3	0,3
Central America 6 (A)	0,3	0,4	n.d.			
South America 1 (A)	n.d.		n.d.		0,1	0,2
South America 2 (A)	n.d.		n.d.		0,1	0,3

(A) = Arabica; (R) = Robusta; n.d. = not detected; St.d. = standard deviation. For each sample several replicates has been effected.

A correspondence between the samples with a high concentration of off-flavours, (especially TCA) and those with OTA contamination (mostly in West and East African coffees) results evident, as can be better seen in Figure 1.

The presence of GEO is evident only in coffees coming from two countries of West Africa, where also the average values of TCA and OTA resulted the highest. Nevertheless, GEO content correlates pretty well (68%) with OTA contamination, even if less significantly than TCA does with OTA (86%).

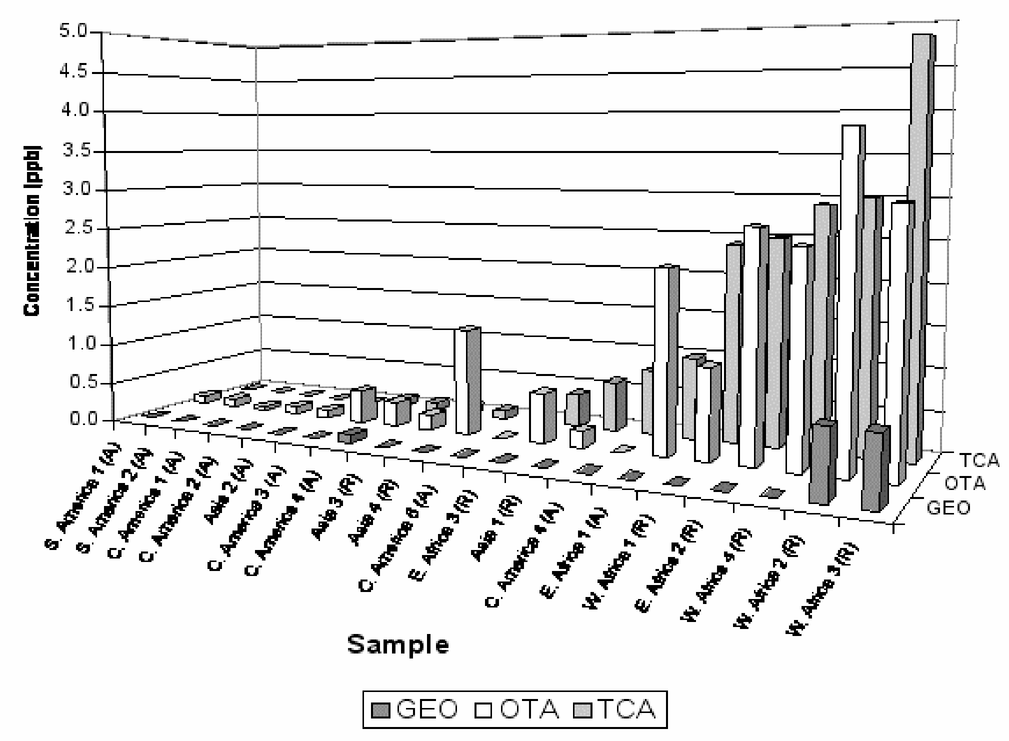

Figure 1. Correlation TCA-GEO-OTA

Effect of decaffeination

The last part of this study regards the effect of methylene chloride extraction on TCA and GEO. The results of the analyses are reported in Table 2: we can see the good extractive effect of the solvent on the off-flavours investigated – especially on TCA – leading the concentrations below the detection limit.

DISCUSSION AND CONCLUSIONS

While in Arabica coffees, especially the ones of American provenience, the concentrations of TCA, GEO and OTA resulted negligible, coffees coming from African countries, nearly all belonging to Robusta species, evidenced the highest concentrations of all contaminants. Interestingly, the comparison of the data obtained provides evidence for the existence of some correlation (up to now only of statistical nature) between the presence of molecules responsible for off-flavours and the presence of OTA, providing a prospective tool for a rough screening of regular green coffee samples against contamination.

No investigation about actual mould infection of the coffee samples was within the scope of this work. Therefore, no causal link can be here suggested between the presence of such different fungal metabolites like TCA, GEO and OTA, and possible interactions among different mycetes. Nevertheless, the possibility that agricultural or post-harvesting phenomena may favour the formation of some complex mould ecosystem should be considered by mycologists as an interesting challenge. Any elucidation of the influence of fungal metabolism on undesirable traits affecting coffee wholesomeness and quality should be more than welcome by both growers and traders.
For the moment being, a pragmatic suggestion could be put forward in order to reduce the impact of obnoxious taints on coffee quality: any measure able to make mould growth difficult, such as improved care on harvesting and thorough drying process, is likely to produce a sounder commodity.

Table 2.

SAMPLE	REGULAR GREEN COFFEE		DECAFFEINATED COFFEE	
	TCA ppb	GEO ppb	TCA ppb	GEO ppb
South America 1 (A)	n.d.	n.d.	n.d	n.d
South America 1 (A)	n.d.	n.d.	n.d	n.d
West Africa 3 (R)	4,3	n.d.	n.d	n.d
West Africa 3 (R)	5,2	n.d.	n.d	n.d
West Africa 2 (R)	4,9	n.d.	n.d	n.d
Central America 4 (A)	0,7	n.d.	n.d	n.d
Asia 1 (R)	n.d.	n.d.	n.d	n.d
Asia 1 (R)	0,7	0,2	n.d	n.d
Asia 2 (A)	n.d.	n.d.	n.d	n.d

(A) = Arabica; (R) = Robusta; n.d. = not detecte. For each sample several replicates has been effected.

The results of the analysis on green decaffeinated coffee samples allow us to verify the good extractive capability of the solvent used in the process - namely methylene chloride - for the molecules subject of this survey, especially TCA. Decaffeination shows to be a process apt to reduce undesirable off-flavours in tainted coffees.

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