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Determination of the Level of Ochratoxin A and The Merchantability Criteria of Ivoirian Green Coffee Intended for Exportation

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Abstract : ***The objective of this study is to follow the evolution of Ochratoxin A levels and merchantability criteria of green coffee beans, in two consecutive seasons, at the port of Abidjan. Thus, 145 green coffee samples, of which 70 were collected during the 2008 campaign and 75 during the 2009 campaign, were analyzed. The merchantability criteria and ochratoxin A (OTA) levels were determined according to the Ivorian standards and the reference method of the European Union regulation respectively. The results indicate average moisture content of green coffee beans of 11.3% and 11.2% respectively for the 2008 and 2009 campaigns. Regarding the classification in grade, no sample belongs to grade 0 (G0) regardless of the campaign. The proportions of coffee grade I (GI) are 16% and 23% respectively for the 2008 and 2009 campaigns. Those of green coffee grade II (GII) are 41% and 37% respectively for the campaigns of 2008 and 2009. The coffee grade III (GIII) has rates of 39% and 28% respectively for the 2008 and 2009 campaigns. The proportions of Coffee Grade IV (GIV) are 4% and 12% respectively for the 2008 and 2009 campaigns. As for the total defects, it has rates of 12.61% and 7.83% respectively for the campaigns of 2008 and 2009. The average OTA concentrations determined in the green coffee batches do not vary statistically for any campaign and values are below 5 µg/kg. This mycotoxin was not detected in 14% of the samples taken during the 2008 campaign against 0% in 2009. In addition, 13% and 9% of the samples taken during the campaigns of 2008 and 2009 respectively have concentrations above 5 µg/kg. Thus special attention should be paid to the treatments of coffee cherries and taking into account the physical characteristics of green coffee grains during the selection of varieties to be popularized.***

Keywords: ochratoxin A, green coffee, merchantability, Côte d'Ivoire

1. Introduction

Coffee is one of the most consumed drinks in the world. Its composition varies according to the variety of coffee, the mode of production of the green coffee beans and the conditions of their roasting. Consequently, the analysis of the constituents of coffee still gives rise to numerous studies, which are justified by the physiological effects of this drink. Several contaminants may be present in the green coffee and eventually end up in the prepared drink. These contaminants include ochratoxin A (OTA), which is a mycotoxin produced by the molds *Aspergillus* and *Penicillium* [1], [2]. Its presence may be of concern because of its toxic or even carcinogenic nature [3]-[7]. The European Commission has set maximum levels of 10 µg/kg and 5 µg/kg respectively for soluble coffee and roasted coffee (grain or ground) through Regulation (CE) 1881/2006 [8]. This standard for roasted coffee also requires control of green coffee, since the mixing of batches contaminated with healthy lots, in order to lower the contamination rate, is prohibited by Regulation (CE) No 466/2001 [9]. The application of these standards could have a significant impact on Côte d'Ivoire's economy, including a loss of market share, a significant reduction in foreign exchange inflows, a significant decline in producers' incomes and a decline in production. At best, Côte d'Ivoire has only fragmentary data on the level of ochratoxin A contamination of its green coffee both in the production areas and in the ports. Thus, it seemed to us necessary to conduct a study on the quality of green coffee grains for export, taking into account the level of ochratoxin A contamination and the physical characteristics. This will be a good contribution to controlling the merchantability and the sanitary value of green coffee exported from Côte d'Ivoire.

2. Material and Method

2.1 Sampling

Sampling consisted of taking samples of 10 kg of green coffee beans destined for export in accordance with Regulation (CE) 401/2006 [10]. Samples were collected from March to July at the port of Abidjan. Thus, in two consecutive campaigns, 145 green coffee samples were taken, including 70 during the 2008 campaign and 75 for the 2009 campaign.

2.2 Determination of the merchantability of green coffee beans

According to the Ivorian regulations [11], [12], the assessment of merchantability includes the moisture content, the particle size or grade of the green coffee and the number of defects defined by Order No. 47/1999 [13]. The moisture content of the green coffee was determined in an oven at 105° C to constant weight [14]. Grading was done using round-hole sieve sets. Green coffees are classified and row into 5 grades (Table 1). Grade 0 (G0) corresponds to the coffee that is retained by the sieve No. 18 with a tolerance of 6% of beans passing through the sieve 18 and 1% at the most at sieve No. 16. The interpretation is the same for all

other grades of coffee [11], [12]. The expertise of each sample was based on Ivorian standards [11], [12]. The defects are counted on an aliquot whose mass is a function of the grade (Table 1) and the defect rate (weight of the defect on weight of the aliquot expressed as a percentage) is determined. All defects were taken into account with an emphasis on broken grains, black grains, indesirable grains and cherries.

Table 1: Ivorian standard for the merchantability of green coffee

Moisture Rate of Sample		
Maximum permissible value		13%
Granulometry or Grade*		
Grade	**Sieve number**	**Tolerance**
Grade 0	18 (7.14 mm)	6% sieve 16 et 1% sieve 14
Grade I	16 (6.35 mm)	6% sieve 14 et 1% sieve 12
Grade II	14 (5.55 mm)	20% sieve 16; 6% sieve 12 et 1% sieve 10
Grade III	12 (4.76 mm)	20% sieve 14 ; 6% sieve 10 et 1% base
Grade IV	10 (3.96 mm)	20% sieve 12 et 6% base
Analysis of Defects		
Grade		**Mass taken (gram)**
Grade 0 (G0)		400
Grade 1 (GI)		360
Grade 2 (GII)		300
Grade 3 (GIII)		230
Grade 4 (GIV)		180

* Any sample that does not meet the grade 4 criteria is out of the ordinary

2.3 Determination of ochratoxin A

2.3.1 Extraction of the ochratoxin A

The entire sample was crushed in a hammer mill to obtain a homogeneous fine grind. In a Nalgene jar containing 15 g of homogenate, 150 mL of aqueous methanol-bicarbonate 1% (m/v; 50:50) were added. The mixture was homogenized by Ultra-Turax for 3 minutes and the homogenate was centrifuged at 5000 rpm for 5 minutes at 4°C. The supernatant was filtered through filter paper into tubes of 25 mL. To 11 mL of filtrate were added 11 mL of saline phosphate buffered (PBS) at pH 7.3. Immunoaffinity columns brand Ochraprep were conditioned with 10 mL of PBS. Purification of 20 mL of the mixture was made on immunoaffinity columns and OTA extraction was performed with two volumes of 1.5 mL of solvent (methanol/acetic acid; 98:2; v/v) at a flow rate of 5 mL/minute. The resulting sample was packed in a chromatographic tube and the analysis for OTA was made by HPLC using the European community regulation [10].

2.3.2 Apparatus

A liquid chromatograph HPLC brand Shimadzu coupled to a fluorescence detector was used and the operating conditions are described in Table 2.

Table 2: HPLC analytical conditions

Precolumn	Shim-pack GVP-ODS 10 × 4.6 mm
Column	Shim-pack GVP-ODS , 250 mm × 4.6 mm
Detector	Fluorescence, λ excitation: 330 nm, λ emission: 460 nm
Mobile phase	Acetonitrile/Water/Acetic acid (99/99/2)
Injected volume	100 µl
Flow rate	1 mL/minute
Column temperature	40°C
Rinsing solvent	Acetonitrile
Analysis duration	12 minutes
Limit of detection (LOD)	0.05 µg/kg
Limit of quantification (LOQ)	0.16 µg/kg
Repeatability (Coefficient of variation ; CV)	1.68%
Reproducibility (Coefficient of variation ; CV)	4%
Extraction yield (EY)	88±0.33%

2.3 Statistical Analysis

Averages were calculated with their standard deviations to assess OTA levels, moisture levels and physical criteria of green coffee. The data were subjected an analysis of variance (ANOVA) with a classification criterion using the SPSS Statistics version 22 software. Averages were compared by the Newman Keuls test at the 5% significance level.

3. Results

3.1 Merchantability of green coffee lots

The average moisture content of green coffee grains is 11.3% and 11.2% respectively for the 2008 and 2009 campaigns. Analysis of variance indicated no significant difference between these values at 5% risk. However, 4% of the samples taken during the 2008 campaign have rates above 13% compared to 0% for those of the 2009 campaign (Table 3).

Regarding the classification in grade, except the proportions of coffee grade 0, all other proportions differ from one season to another. Indeed, no sample belongs to grade 0 regardless of the campaign. The proportions of coffee grade I are 16% and 23% respectively for the 2008 and 2009 campaigns. Those of coffee grade II are 41% and 37% respectively for the 2008 and 2009 campaigns. Grade III coffee has rates of 39% and 28% respectively for the 2008 and 2009 campaigns. The proportions of grade IV coffee are 4% and 12% respectively for the 2008 and 2009 campaigns (Table 3).

As for the total defects, they have variable rates from one season to another and these rates are 12.61% and 7.83% respectively for the campaigns of 2008 and 2009. Broken grains content is 2.05% and 0.42% respectively for the 2008 and 2009 campaigns and a significant difference is observed between these values. Rates of cherries and black grains do not vary from one season to another and remain below 0.5%.

The indesirable grains have respective rates of 9.61% and 6.37% for the 2008 and 2009 campaigns and a significant difference is observed between these values. Green coffee rates are higher in 2009 campaign (92.16%) than in 2008 (87.39%, Table 3).

Table 3: Merchantability of green coffee lots

Moisture content*			
Campaigns	**Average (%)**	**[Min-Max]**	**Percentage samples H>13**
2008	11.3±1.0a	10.0-14.2	4
2009	**11.2±0.85a**	**10.0-13.0**	**0**
GRADE OR GRANULOMETRY (percentage)			
Grade		**Campaign 2008**	**Campaign 2009**
Grade 0 (G0)		0	0
Grade 1 (GI)		16	23
Grade 2 (GII)		41	37
Grade 3 (GIII)		39	28
Grade 4 (GIV)		4	12
Proportion of Defects and Green Coffee (percentage)**			
Designation		**Campaign 2008**	**Campaign 2009**
Broken grains		2.05±1.25a	0.42±0.37b
Cherries		0.16±0.14a	0.21±0.16a
Black grains		0.12±0.11a	0.14±0.10a
Indesirables grains		9.61±2.88a	6.37±4.67b
Total defects		12.61±3.77a	7.83±5.53b
Green coffee		87.39±3.77b	92.16±5.53a

The averages of the same column (*) or line (**) bearing the same letter do not show a significant difference at the risk p <0.05.

3.2 Ochratoxin A concentration of the green coffee lots

The average OTA concentrations determined in the green coffee lots do not vary from one campaign to another and values are below 5 µg/kg. This mycotoxin was not detected in 14% of the samples taken during the 2008 campaign against 0% for that of 2009. In addition, 13% of the samples collected during the 2008 campaign have concentrations greater than 5 µg/kg compared to 9% for 2009 (Table 4).

Table 4: Levels of ochratoxin A contamination of the lots of green coffee

OTA concentration			Percentage	
Campaigns	Average (µg/kg)	[Min-Max]	< LOD	> 5 µg/kg*
2008	2.35±1.86a	0.05-17.58	14%	13%
2009	3.25±2.36a	0.12-17.18	0%	9%

The averages of the same column bearing the same letter do not show a significant difference at the risk p <0.05.
*LMR fixée pour le café torréfié par la commission européenne

3.3 Merchantability of green coffee according to different grades

The average moisture content of different grades of green coffee does not vary significantly regardless of grade and campaign. They vary between 11.2% and 11.6% for the 2008 campaign and between 11.0% and 11.4% for the 2009 campaign. Broken grains rates ranged from 1.61% to 2.47% for the 2008 campaign and 0.06% to 0.77% for the 2009 campaign. During the 2008 campaign, no significant difference was observed between values regardless of grade. While, for the 2009 campaign, the high rates are obtained with coffee GIII (0.77%) and GIV (0.71%). In addition, regardless of grade, high broken grains was determined during the 2008 campaign. As for cherries and black grains, the average rates do not vary significantly regardless of the grade and the campaign. Cherries rates vary between 0.03% and 0.26% for the 2008 campaign and between 0.16% and 0.33% for the 2009 campaign. Those of black grains vary between 0.10% and 0.18% for the 2008 campaign and between 0.10% and 0.21% for the 2009 (Table 5).

As regards the indesirable grains and the total defects, they have respective average rates varying from 8.38% to 10.04% and from 11.69% to 13.71% for the 2008 campaign and from 3.02% to 9.25% and from 4.00% to 12.67% for that of 2009. During the 2008 campaign, no significant difference was observed in terms of indesirable grains or total defects, regardless of the grade of coffee. In contrast, during the 2009 campaign, high levels of indesirable grains and total defects were determined in GIII and GIV coffee (Table 5). Indesirable grains levels and total defects of GI and GII coffees determined in the 2008 campaign are higher than in the 2009 campaign. The proportions of green coffee do not differ significantly regardless of the grade and the campaign. However, a significant difference is observed between the campaigns for the GI and GII coffees and the high proportions are determined during the 2009 campaign. In addition, the proportions of green coffee are higher for GI and GII coffees than for GIII and GIV coffees for 2009 campaign. The different proportions of green coffee range from 86.29% to 88.31% for the 2008 campaign and from 87.33% to 96.00% for the 2009 campaign (Table 5).

3.4 Ochratoxin A contamination of green coffee according to the different grades

The average concentrations of ochratoxin A of the green coffee grades ranging from 1.36 µg/kg and 4.44 µg/kg for the 2008 campaign and between 2.11 µg/kg and 5.06 µg/kg for the 2009 campaign. The statistical analysis of the variances revealed no significant difference between the averages regardless of the grade and the campaign at the risk 5% (Table 5).

3.5 Monthly evolution of moisture, broken grains, total defects and ochratoxin A

The moisture content has a relatively homogeneous evolution (11.1% to 11.4%) because it varies very little from one month to another of the campaigns. On the other hand, broken grains rates, total defects and ochratoxin A concentration have evolved in 2 phases. It is observed an ascending phase from March to May followed by a downward phase from May to July. A peak is reached by each of these parameters during the month of May and equal to 1.45%, 11.1% and 3.44 µg/kg respectively for broken grains, total defects and ochratoxin A concentration (Figure 1).

Table 5: Distribution of merchantability parameters and contamination of ochratoxin A according to the grade

Moisture (percentage)						p
Campaigns	**G0**	**GI**	**GII**	**GIII**	**GIV**	
2008	-	11.3±1.1aα	11.4±1.0aα	11.2±1.0aα	11.6±1.1aα	0.888
2009	-	11.1±0.9aα	11.2±0.8aα	11.0±0.8aα	11.4±1.1aα	0.729
Signification (p)	-	0.683	0.307	0.371	0.960	-
Broken grains (percentage)						**P**
Campagnes	**G0**	**GI**	**GII**	**GIII**	**GIV**	
2008	-	1.61±0.78aα	1.82±1.05aα	2.47±1.55aα	2.10±0.58aα	0.503
2009	-	0.06±0.05bβ	0.30±0.18bβ	0.77±0.65aβ	0.71±0.34aβ	0.000
Signification (p)	-	0.000	0.000	0.009	0.008	
Cherries (percentage)						**P**
Campagnes	**G0**	**GI**	**GII**	**GIII**	**GIV**	
2008	-	0.26±0.17aα	0.15±0.09aα	0.13±0.04aα	0.03±0.01aα	0.533
2009	-	0.33±0.10aα	0.16±0.09aα	0.19±0.12aα	0.22±0.11aα	0.527
Signification (p)	-	0.482	0.404	0.795	0.089	
Black grains (percentage)						**P**
Campagnes	**G0**	**GI**	**GII**	**GIII**	**GIV**	
2007-2008	-	0.10±0.08aα	0.11±0.08aα	0.14±0.09aα	0.18±0.10aα	0.441
2008-2009	-	0.10±0.07aα	0.10±0.08aα	0.21±0.11aα	0.20±0.10aα	0.115
Signification (p)	-	0.979	0.419	0.480	0.357	
Indesirable grains (percentage)						**P**
Campagnes	**G0**	**GI**	**GII**	**GIII**	**GIV**	
2008	-	9.40±2.02aα	9.43±3.08aα	10.04±3.07aα	8.38±2.48aα	0.668
2009	-	3.02±1.29bβ	5.52±2.81bβ	9.25±4.63aα	8.81±2.64abα	0.000
Signification (p)	-	0.000	0.000	0.571	0.920	
Total defects (percentage)						**P**
Campagnes	**G0**	**GI**	**GII**	**GIII**	**GIV**	
2008	-	11.69±2.98aα	11.95±3.55aα	13.71±4.19aα	12.38±3.88aα	0.700
2009	-	4.00±1.60bβ	6.33±2.04bβ	10.97±4.34aα	12.67±3.29aα	0.000
Signification (p)	-	0.000	0.000	0.129	0.896	
Green coffee (percentage)						**P**
Campagnes	**G0**	**GI**	**GII**	**GIII**	**GIV**	
2008	-	88.31±2.98aβ	88.05±3.55aβ	86.29±4.19aα	87.62±3.88aα	0.700
2009	-	96.00±1.60aα	93.65±2.04aα	89.03±4.34bα	87.33±5.29bα	0.000
Signification (p)	-	0.000	0.000	0.129	0.896	
Ochratoxin A (µg/kg)						**P**
Campaigns	**G0**	**GI**	**GII**	**GIII**	**GIV**	
2008	-	2.25±1.88aα	1.36±0.46aα	3.23±2.62aα	4.44±2.19aα	0.124
2009	-	2.71±1.09aα	2.11±0.92aα	4.33±2.05aα	5.06±3.33aα	0.287
Signification (p)	-	0.665	0.122	0.515	0.881	

The averages followed by the same letter do not show a significant difference at the risk p <0.05. The letters a and b denote an online comparison and the letters α and β a column comparison.

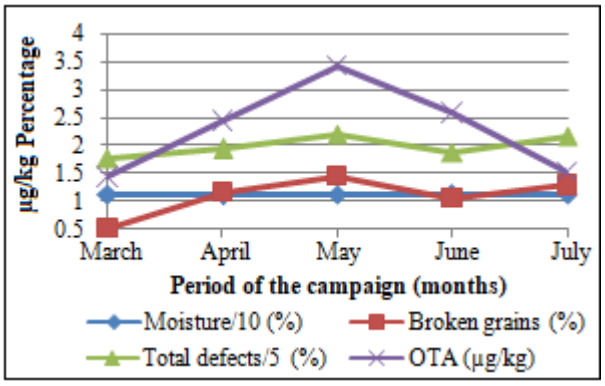

Figure 1: Monthly evolution of moisture, broken grains, total defects and ochratoxin A concentration

3.6 Monthly evolution of grades of green coffee

Coffee grade I has a sawtooth evolution with 2 peaks reached in April (30%) and June (30%). On the other hand, the other grades (GII, GIII and GIV) have an evolution in 2 phases. There is an ascending phase from March to April for grade II coffee and from March to May for grade III and IV coffees. The peaks are reached in April (41%) for grade II and in May (30% and 50% respectively) for grades III and IV (Figure 2).

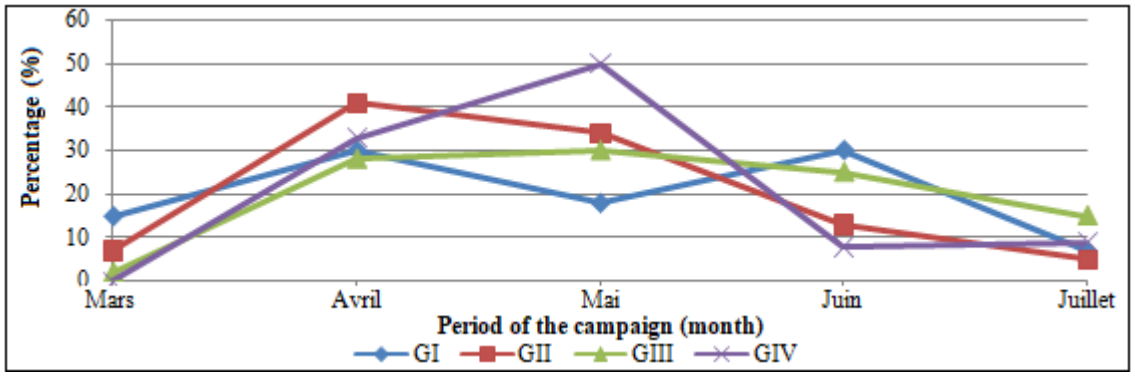

Figure 2 : Monthly evolution of the different grades of green coffee

4. Discussion

The green coffee intended for export is well dried whatever the campaign and the grade. However, this stage of drying was well conducted during the 2009 campaign than that of 2008. In fact, 4% of the samples taken during the 2008 campaign have rates higher than 13%, which is the maximum value allowed in green coffee for export [11], [15], [16]. Whatever the campaign, the average moisture content is identical to the 11.07% determined by Dembelé et *al*. [17]. This situation could result from the control of green coffee drying by producers and exporters. According to the work of Kouadio et *al*. [18], cherries drying is an important step in the post-harvest treatments of coffee by the dry method which is the technique used by Ivorian producers.

The particle size of green coffee grains is an important factor in the quality of coffee. In Côte d'Ivoire, this particle size is evaluated through the grade of green coffee and decreases from grade 0 to grade IV [11], [16]. Grade 0 coffee was not found at the port regardless of the campaign. This result differs from that of Dembelé et *al*. [17] who determined 4% of grade 0 coffee during their study. However, coffee grade II has the highest proportion regardless of the campaign and this result corroborates that of Dembelé et *al*. [17]. Thus, the Ivoirian coffee destined for export is mostly of average size (5.55 mm to 6.35 mm) with peaks observed during the months of April (grades I and II) and June (grade I). The small grains (grades III and IV) represent about 40% of the samples with peaks observed during the month of May. According to the work of Fallo and Ngongang-Nono [19], this variability in the size of coffee grains is related to the variety of coffee. In their study, the 2 varieties from Côte d'Ivoire have coffee grade I levels ranging from 16.44% to 57.75% [19]. The physical characteristics of green coffee including grain size or grade (grades 0 to IV) is an important criterion for marketing green coffee [19], [20], [16]. Thus, in Côte d'Ivoire, emphasis should be placed on the physical characteristics of coffee grains when selecting varieties to be popularized.

Post-harvest treatments, such as drying, shelling and storage, affect the quality of green coffee because they can cause some defects [21], [20]. According to the Codex Alimentarius [22], defects represent indesirable particles that may include various types of grains or parts of grains, fruit tissues and foreign materials often found in green coffee lots. While defective or indesirable grains usually result from inadequate treatment, insect attack or poor weather [22]. Post-harvest grain treatments were relatively well conducted by producers during the 2009 campaign. Because the levels of indesirable grains and total defects are low. This annual variability in post-harvest processing of coffee grains is more pronounced with peaks in broken grains and total defects observed during the month of May. This could be explained by the beginning of the rainy season synonymous with the occupation of the producers by farm work other than the processing of coffee cherries.

Ivorian green coffee for export complies with the European standard for ochratoxin A contamination of roasted and roasted ground coffee grains [8]. Indeed, all the average concentrations are below 5 µg/kg which is the maximum level of ochratoxin A authorized by the European Union in ground roasted and roasted coffee grains [8]. There is still no European standard for ochratoxin A contamination of green coffee [22]. The average contamination level of ochratoxin A of Ivorian green coffee, intended for export, did not vary significantly from 2008 to 2009. However, the proportion of samples with concentrations greater than 5 µg/kg is higher in the 2008 campaign (13%) than in 2009 (9%). This could be explained by the reduction in the rate of coffee bean defects during the 2009 campaign, particularly the broken grains. Dembele et *al*. [17] showed in their study a high level of ochratoxin A contamination in broken grains lots (39.7 µg/kg). These authors also showed a positive correlation between broken grains and ochratoxin A concentration with a Pearson coefficient of 0.531 [17]. The contamination of green coffee with ochratoxin A is greater during the month of May and coincides with the high levels of broken grains and total defects. Thus, an improvement in the post-harvest treatments of coffee cherries during the month of May by producers could reduce the occurrence of broken grains and total defects in Ivorian green coffee. As a consequence, this practice could also reduce the concentration of ochratoxin A.

5. Conclusion

This study allowed to have information on the physical characteristics and the contamination level of ochratoxin A in green coffee sampled at the port of Abidjan. Green coffee grains are mostly of medium size and their post-harvest treatments by producers vary from one season to another. The average concentrations of ochratoxin A in green coffee remain below the standard set by the European Union for roasted coffee. This study nevertheless reveals that approximately 9% to 13% of the samples are non-compliant with this standard. It is therefore important to pay particular

attention to the treatments of coffee cherries and also to take into account the physical characteristics of green coffee grains during the selection of varieties to be popularized. This study is expected to extend to green coffee production areas in Côte d'Ivoire.

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