



# Differentiation of degrees of ripeness of *Catuai* and *Tipica* green coffee by chromatographical and statistical techniques



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## ABSTRACT

The quality of green coffee is influenced by the degree of ripeness of the fruit at harvest. The aim of this study was to identify chemical markers differentiating between degrees of ripeness. Two coffee varieties, *Catuai* and *Tipica*, from the same farm were analysed using the following parameters and methods: caffeine and chlorogenic acid content using high-performance liquid chromatography (HPLC), sucrose content using hydrophilic interaction chromatography, high-molecular weight fraction (HMW) using high-performance size-exclusion chromatography (HPSEC) and volatile compounds using headspace solid phase micro extraction gas chromatography/mass spectrometry. The best method for differentiating between degrees of ripeness was found to be principal component analysis (PCA) based on HPLC data. HPSEC showed differences in the HMW fraction for different degrees of ripeness and both coffee varieties. Volatile profiles allowed separation of both varieties; yet, except for ripe *Catuai*, no separation was achieved for the degree of ripeness.

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## 1. Introduction

With the growing interest in speciality and single origin, high quality coffees, more and more focus is being placed on the objective evaluation of the quality of the raw material itself, green coffee. In combination with efforts in consumer countries to refine the roasting, grinding and brewing processes, objective evaluation of the raw material has already led to improvements in cup quality at the consumer end of the value chain, and further improvements are expected in the future. In order to produce and source high quality green coffee, more knowledge of how to objectively assess the quality of coffee prior to roasting is required.

In coffee trading, certain parameters, such as bean size, shape, colour, origin and crop-year, are often used as quality criteria. It is also well known that defects in green coffee beans have a negative impact on cup quality, and the identification and classification of such defects is an integral part of quality grading. Coffee producing countries have each developed their own defect classification schemes that are based on visual parameters. Final assessment of the quality of a coffee is usually performed by roasting, grinding, brewing and tasting a sample, a process called “cupping”. It should be noted that such green coffee quality evaluation processes are highly subjective. Furthermore, many high quality specialty coffees

have become increasingly free of defects, meaning quality evaluation schemes that are based on counting specific defects are of little use for this segment of the market.

A very critical quality indicator is the degree of ripeness of the harvested and processed coffee fruits. Many large scale production farms do not sort their crops, meaning that coffee beans of widely varying degrees of ripeness are picked simultaneously resulting in a lower cup quality. There is, however, an increasing number of farms that specialise in high quality coffee. These farms have established harvesting and post-harvesting processes, including the manual harvesting of coffee cherries, to ensure that only fully ripe fruits are picked. In combination with a thorough sorting of the green beans, this allows the producer to deliver a premium quality coffee.

To support the trend towards premium quality coffee, it is important to develop evaluation schemes that are more appropriate for this “defect-free” segment, and that can differentiate between coffees harvested at different degrees of ripeness. So far, little is known about the changes in chemical composition of green beans during the ripening of the fruits and how green bean composition can be analytically related to the quality of the cup of coffee. Most of the studies to date have focused on markers for defective beans (immature, overripe). Different methods, such as direct injection ESI-MS of a coffee extract (Mendonca, Franca, Oliveira, & Nunes, 2008), levels of amines in the beans (Vasconcelos, Franca, Gloria, & Mendonca, 2007) and volatile fingerprinting

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(Toci & Farah, 2008, 2014) have been used to distinguish defective from non-defective coffee beans. A study found that immature beans can be differentiated from mature (ripe) beans using diffuse reflectance infrared spectroscopy (Craig, Franca, & Oliveira, 2012). Direct injection electrospray ionisation mass spectrometry on methanol extracts of green and roasted beans has also been used to distinguish between immature, ripe and overripe beans (Amorim et al., 2009). The main differences between the beans were in the fatty acid content and the drop in di- and trimeric chlorogenic acids (CGAs) signal intensities. Slight differences have also been found in lipid content between immature and ripe coffee beans (Jham, Velikova, Muller, Nikolova-Damyanova, & Cecon, 2001). It has been found that the chlorogenic acid content in unprocessed coffee beans decreases with maturation of the coffee fruit, and that there is a difference between the ripe (pink) and fully ripe fruit (Koshiro et al., 2007). Elemental composition has also been investigated (Valentin & Watling, 2013), but no differences were found with respect to degrees of ripeness.

The aim of the presented work was to search for differences in chemical composition between coffee beans harvested at different degrees of ripeness, using wet-processed green coffee beans from a single origin that are free from defects. The chosen stages of ripeness were all in the range of normal commercial coffee qualities. A range of analytical methods was optimised and developed to analyse selected parameters: chlorogenic acid profile, volatile profile, caffeine, sucrose content and high-molecular weight (HMW) part of the size exclusion chromatogram.

## 2. Materials and methods

### 2.1. Coffee beans

Green coffee beans were obtained from the Finca SHANTI of Munaipata Café de Altura S.A., Coroico, Bolivia (16° 13' 05" S, 67° 43' 25" W, elevation 1700–1880 m). The coffee plants had been exposed to identical soil and sunshine conditions. Fruits from two varieties of Arabica, *Tipica* and *Catuai*, were harvested at three different stages of ripeness, namely unripe, half-ripe and ripe, as shown in Fig. 1. Unripe fruits were those that had just started to show a red colour on an otherwise mostly green fruit, half-ripe fruits were the opposite and were mostly completely light red in colour with some remaining green spots and ripe fruits were completely deep red in colour. The raw coffee beans were obtained from the fruits by the wet-process post-harvest treatment. All samples were free of defects.

### 2.2. Chemicals

Methanol and acetonitrile were obtained from Sigma-Aldrich and were of HPLC gradient grade, sodium phosphate and phosphoric acid were reagent grade from Sigma-Aldrich (Buchs SG,

Switzerland) and formic acid was from Fluka, eluent additive LC-MS grade. Caffeine and sucrose standards were obtained from Fluka, 3-caffeoyl quinic acid (3-CQA), 4-caffeoyl quinic acid (4-CQA) and 5-feruoyl quinic acid (5-FQA) from Sigma-Aldrich and 5-caffeoyl quinic acid (5-CQA) from Acros Organic (Geel, Belgium). Millipore grade water was used.

### 2.3. Preparation of extracts

Before each extraction, the green coffee beans were frozen in liquid nitrogen and ground to a fine powder using an MM 400 ball mill (Retsch, Germany) for 90 s at 30 Hz. Two types of extracts were prepared for analysis of the non-volatile composition of green coffee, a methanol and a water extract. Methanol extracts were prepared by Soxhlet extraction using a Büchi extraction system B-811 (Büchi, Switzerland). Soxhlet extraction was carried out in four cycles, extracting 2 g of ground coffee powder with 100 ml of methanol, with heating set to 14 (arbitrary value), followed by a 10 min reflux washing step. The water extracts were prepared using hot water; 3 g of the green coffee powder was infused in 100 ml of water at 92 °C, stirred for 5 min and filtered using a paper filter. All samples were prepared in triplicate.

### 2.4. Reverse-phase high-performance liquid chromatography

The methanol extracts of the green coffee were analysed on an HPLC-MS system (Agilent 1200 HPLC with 6130 quadrupole MS). Separation was carried out on a Poroshell 120 EC-C18 2.7 µm, 2.1 × 100 mm column (Agilent) and a corresponding pre-column, with a flow rate of 0.3 ml/min and the following elution of linear gradients of the mobile phases A (water: methanol 90: 10 (v/v) with 0.1% formic acid) and B (water: methanol 5: 95 (v/v) with 0.1% formic acid): 0–1 min 10% B, 5 min 20% B, 12 min 40% B, 18 min 70% B and 19 min 10% B. The injection volume was 2 µl and the post run equilibration time was 6 min. Both MS and UV/VIS detection were used. MS and comparison of retention times to standards were used for identification purposes. Quantification of compounds was carried out by a UV/VIS detector by integrating peak areas at 325 nm for CGAs and 275 nm for caffeine. CGAs were quantified as 5-CQA equivalents. Samples for analysis and for standards were prepared by pipetting 1 ml of the methanol extract or 1 ml of the solution of the standard in methanol, respectively, into 10 ml volumetric flasks, which were then filled to volume with water. Samples were filtered prior to injection using 0.45 µm PET syringe filters (Macherey-Nagel, Germany).

### 2.5. High-performance size-exclusion chromatography

High-performance size-exclusion chromatography was performed based on a modification of a previously described method (Smrke, Opitz, Vovk, & Yeretzyan, 2013). Briefly, two columns were used in series, first a SupermultiporePW-N 4 µm, 6 × 150 mm and



Fig. 1. Photographs of *Catuai* coffee fruits in the three different degrees of ripeness used in this study, before processing. The degrees of ripeness were described as unripe (a), half-ripe (b) and ripe (c).

secondly a SupermultiporePW-M 5  $\mu\text{m}$ , 6  $\times$  150 mm column, both from TSKGel (Tosoh Bioscience, Stuttgart, Germany). The mobile phase was a 0.1 M aqueous solution of sodium phosphate with pH adjusted to 7.0 with phosphoric acid. A flow rate of 0.4 ml/min and an injection volume of 5  $\mu\text{l}$  were used. Detection was performed by UV/VIS at 210 nm, 280 nm and 325 nm. At 210 nm and 280 nm, the total high-molecular weight (HMW) fraction of the chromatogram was integrated from a retention time of 11.2 min to 24.5 min and at 325 nm the low-molecular weight (LMW) peak was integrated (Fig. 2). Water extracts were filtered using 0.45  $\mu\text{m}$  PET syringe filters (Macherey-Nagel) prior to injection into the HPSEC system.

## 2.6. Determination of sucrose content

The sucrose content was determined by HPLC using an amino-propyl silica column, Carbohydrate 5  $\mu\text{m}$ , 4.6  $\times$  150 mm, (Agilent Technologies, Switzerland). Acetonitrile:water 75:25 (v/v) was used as the eluent, with a flow rate of 1.5 ml/min and an injection volume of 50  $\mu\text{l}$ . A refractive index detector (Agilent) was used. The run time was 10 min. Samples for sucrose determination were prepared by mixing 2.5 ml of water based green coffee extracts with 7.5 ml of acetonitrile. The samples were filtered prior to injection with 0.45  $\mu\text{m}$  PET syringe filters (Macherey-Nagel).

## 2.7. Volatile profiles of green coffee beans

Volatile profiles of whole green coffee beans were measured using headspace solid phase micro extraction gas chromatography-mass spectrometry (HS SPME GC/MS). Five replicates of whole green coffee beans were weighed in SPME vials ( $m = 4.00 \text{ g} \pm 0.07 \text{ g}$ ) and the headspace was purged with nitrogen

before closing the vials. A poly-dimethylsiloxan/divinylbenzene (PDMS/DVB) SPME fibre with a 65  $\mu\text{m}$  thick film (Supelco, Sigma-Aldrich Chemie GmbH, Switzerland) and a DB-WAX (30 m  $\times$  250  $\mu\text{m}$   $\times$  0.25  $\mu\text{m}$ ) column (Agilent Technologies, Switzerland) were used. The SPME parameters (Gerstel, Switzerland) were as follows: incubation time 10 min, agitating at 250 rpm; extraction time 30 min at 50  $^{\circ}\text{C}$ , pre-run bakeout 250  $^{\circ}\text{C}$  for 6 min. The GC-MS parameters (7890A/5975C, Agilent Technologies, Switzerland) were: 37  $^{\circ}\text{C}$  for 1 min, 4  $^{\circ}\text{C}/\text{min}$  to 100  $^{\circ}\text{C}$ , 10  $^{\circ}\text{C}/\text{min}$  to 170  $^{\circ}\text{C}$ , 3  $^{\circ}\text{C}/\text{min}$  to 185  $^{\circ}\text{C}$  and 10  $^{\circ}\text{C}/\text{min}$  to 220  $^{\circ}\text{C}$ , splitless mode, flow 1 ml/min, EI source 70 eV, 230  $^{\circ}\text{C}$ , detector 150  $^{\circ}\text{C}$ . Data analysis and identification of the compounds was performed using the MSD Chemstation software (Version G1701 EA E.02.00.493, Agilent Technologies, Switzerland) and the NIST08 spectrum database. Chemical identification was performed by comparing the MS spectra to the database, the most intensive fragment ion was used for quantification.

## 2.8. Statistical analysis

Statistical data analysis was performed using the R program package (RStudio, Version 0.97.551, R-3.0.2). Principal component analysis (PCA; *prcomp*, based on singular value decomposition) was performed on centre-scaled data.

## 3. Results and discussion

### 3.1. Caffeine, chlorogenic acid and sucrose content

During method optimisation, columns with different reverse phase sorbents (pentafluorophenyl, C18 endcapped and C18 core-shell) were evaluated, using either methanol or acetonitrile eluents. A common problem was the separation of caffeine from 5- and 4-CQA. Methanol was, in general, a more selective eluent than acetonitrile. Only the final method using the Poroshell column was able to provide sufficient separation between the CGAs and caffeine. A typical green coffee reverse phase HPLC chromatogram is shown in Fig. 2a. The newly developed method can also easily be adapted to create a rapid method for analysis of caffeine and CGAs in roasted coffee. The very low amount of sample that was loaded on the column also prolonged pre-column life and no sample pre-treatment was required.

The content of the selected CGAs and caffeine in the green coffee beans are presented in Table 1. The content of caffeine and the total amount of CGAs are higher for the *Tipica* than the *Catuai* beans. By comparing the degrees of ripeness, the concentration of 3-CQA increased for both coffee varieties from unripe to ripe beans. In contrast, the 5-CQA content decreased for the *Catuai* variety from unripe to ripe beans (Table 1). In addition to the analysis with RP-HPLC, the total amount of CGAs was also estimated from HPSEC chromatograms based on a previously published method (Smrke et al., 2013).

HPSEC yielded results that showed higher total quantities of the CGAs compared to the results from RP-HPLC (by about 18%). These differences might have been caused by overestimation of the CGA content in HPSEC due to insufficient peak separation (hence it is only an estimation of the total CGAs) or by degradation of the CGAs during the Soxhlet extraction. Another factor to highlight is the extraction efficiency from very hard green coffee beans, as water extraction from green coffee beans is dependent on the degree of grinding. For a good extraction efficiency, a fine ground of green coffee is important. The samples for RP-HPLC and HPSEC were both ground in the same way, but extracted via different processes. Together with the differences in chromatography this probably explains the differences in the absolute values.

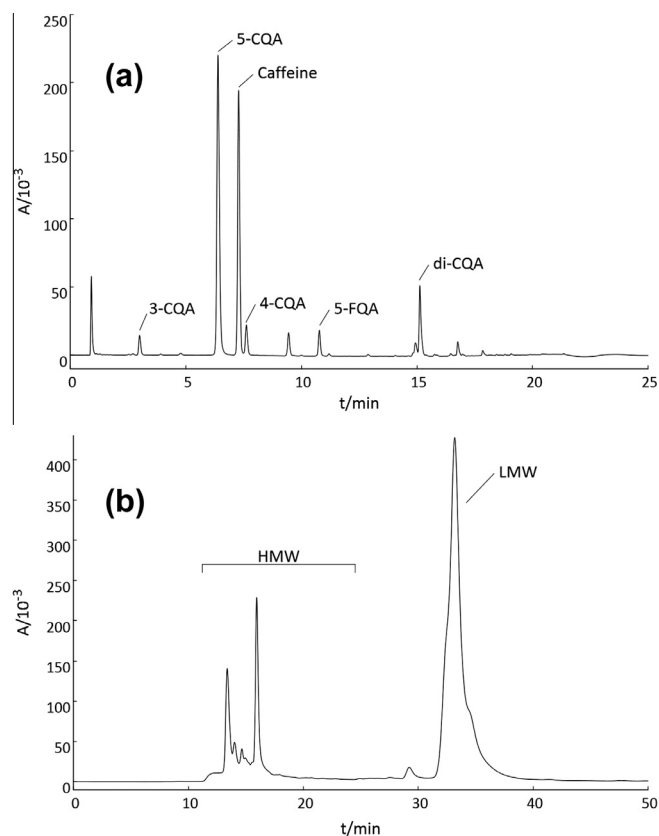


Fig. 2. Chromatograms of *Catuai* ripe green coffee bean extracts, separated by RP-HPLC, detection at 275 nm (a) and HPSEC, detection at 210 nm (b).

**Table 1** Caffeine, chlorogenic acid, sucrose and water content and peak areas of HMW fraction determined for *Tipica* and *Catuai* green coffee beans. The values (mean  $\pm$  standard deviation,  $n = 3$ ) are percentages of total bean weight unless otherwise noted.

| Coffee variety | Degree of ripeness | Water content    | HILIC           | C18 reverse phase high-performance liquid chromatography |                 |                 |                 |                 |               | High-performance size-exclusion |                 |                 |
|----------------|--------------------|------------------|-----------------|--|-----------------|-----------------|-----------------|-----------------|---------------|---------------------------------|-----------------|-----------------|
|                |                    |                  |                 | Sucrose  | 3-CQA           | 4-CQA           | 5-CQA           | 5-FQA           | di-CQA        | Caffeine                        | Total CGA       | HMW A280 (A.U.) |
| <i>Catuai</i>  | Unripe             | 9.07 $\pm$ 0.14  | 7.94 $\pm$ 0.16 | 0.16 $\pm$ 0.01  | 0.28 $\pm$ 0.01 | 0.27 $\pm$ 0.01 | 0.74 $\pm$ 0.04 | 1.01 $\pm$ 0.05 | 4.5 $\pm$ 0.2 | 5.29 $\pm$ 0.03                 | 14056 $\pm$ 402 | 1267 $\pm$ 7    |
|                | Half-ripe          | 9.2 $\pm$ 0.4    | 8.03 $\pm$ 0.05 | 0.17 $\pm$ 0.01  | 0.3 $\pm$ 0.02  | 0.26 $\pm$ 0.02 | 0.69 $\pm$ 0.03 | 1.00 $\pm$ 0.04 | 4.4 $\pm$ 0.3 | 5.29 $\pm$ 0.08                 | 14148 $\pm$ 202 | 1243 $\pm$ 13   |
|                | Ripe               | 11.13 $\pm$ 0.17 | 7.04 $\pm$ 0.07 | 0.17 $\pm$ 0.01  | 0.28 $\pm$ 0.02 | 0.24 $\pm$ 0.02 | 0.69 $\pm$ 0.05 | 0.97 $\pm$ 0.04 | 4.1 $\pm$ 0.3 | 4.85 $\pm$ 0.03                 | 13663 $\pm$ 166 | 1182 $\pm$ 12   |
| <i>Tipica</i>  | Unripe             | 8.6 $\pm$ 0.7    | 7.62 $\pm$ 0.14 | 0.19 $\pm$ 0.01  | 0.33 $\pm$ 0.02 | 0.26 $\pm$ 0.01 | 0.75 $\pm$ 0.04 | 1.04 $\pm$ 0.06 | 4.8 $\pm$ 0.3 | 5.4 $\pm$ 0.06                  | 12431 $\pm$ 138 | 1362 $\pm$ 32   |
|                | Half-ripe          | 9.72 $\pm$ 0.17  | 7.54 $\pm$ 0.15 | 0.20 $\pm$ 0.02  | 0.33 $\pm$ 0.04 | 0.26 $\pm$ 0.02 | 0.73 $\pm$ 0.06 | 1.07 $\pm$ 0.06 | 4.7 $\pm$ 0.4 | 5.41 $\pm$ 0.08                 | 12562 $\pm$ 182 | 1367 $\pm$ 20   |
|                | Ripe               | 9.85 $\pm$ 0.06  | 7.55 $\pm$ 0.12 | 0.21 $\pm$ 0.02  | 0.34 $\pm$ 0.03 | 0.26 $\pm$ 0.02 | 0.73 $\pm$ 0.04 | 1.07 $\pm$ 0.05 | 4.6 $\pm$ 0.3 | 5.41 $\pm$ 0.12                 | 12401 $\pm$ 137 | 1303 $\pm$ 7    |

Sucrose content was also measured in the water extracts of green coffee (Table 1). Besides sucrose, both glucose and fructose are present in green coffee (Knopp, Bytof, & Selmar, 2006; Murkovic & Derler, 2006), however in much lower concentrations than sucrose. The HILIC separation method, based on an aminopropyl silica column with refractive index detection, was only sufficient to determine sucrose concentrations, since the low concentrations of fructose and glucose that were measured overlapped with the CGAs. A sucrose content of 7–8% was found in both coffees, which agrees with previously published data (Knopp et al., 2006). Some differences in sucrose content were observed for the different degrees of ripeness; the unripe and half-ripe *Catuai* samples had the highest content, while ripe *Catuai* beans had the lowest. There was no difference between the degrees of ripeness for the *Tipica* beans.

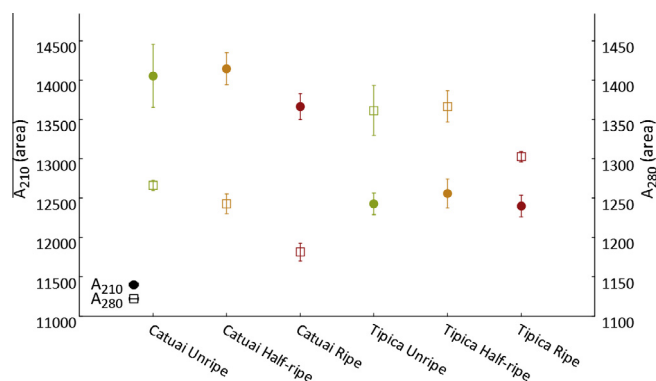
The water content of the green beans was measured to see if it could have an impact on the final measurements. The water content in the ripe *Catuai* beans was considerably higher than in the other beans (Table 1). The difference is yet not sufficient to explain the lower values obtained for all the other compounds (caffeine, sucrose, 5-CQA) that were analysed. Therefore, the values presented in Table 1 have not been corrected for the water content.

### 3.2. High-performance size-exclusion profile

In HPSEC, the focus was placed on the high molecular weight (HMW) part of the chromatograms. The areas of the HMW chromatograms (Fig. 2b) were integrated at 210 nm and 280 nm detection wavelengths to estimate the total UV absorbing water soluble HMW fraction in each sample. The largest differences found were between the two different varieties *Catuai* and *Tipica*. There were profound differences in their compositions: at 210 nm, *Catuai* shows a larger area for the HMW fraction, whereas *Tipica* shows a larger area at 280 nm. The results at 280 nm are particularly interesting; differences between the degrees of ripeness are visible for both coffee varieties, and clearly lower values were measured for the ripe beans than for the unripe and half-ripe ones (Fig. 3).

### 3.3. Volatile profile

The volatile profile of green coffee was investigated by analysing both the headspace of finely ground green coffee as well as of whole green beans. It was found that analysing the headspace above whole green beans is more reproducible than doing the same with ground green coffee. It is likely that volatile oxidation products of green coffee beans occurred with different intensities for different ground replicates and some compounds were even



**Fig. 3.** Peak areas of the HMW fraction of green coffee extracts separated by HPSEC. Two wavelengths were used for detection, 210 nm ( $A_{210}$ ) and 280 nm ( $A_{280}$ ). Error bars show standard deviation,  $n = 3$ .

absent from certain chromatograms. There was no observable systematic trend to these differences in the ground green samples, such as stabilisation of headspace over a time period or degradation with time, therefore sampling above whole beans was used. This not only simplified the analysis of the sample, but also eliminated a processing step (grinding) that may have introduced some variance between the replicates (e.g. particle size distributions) and potentially masked small but real differences in the compositions of the different samples.

In total, 68 compounds were identified and peak areas were integrated. Three different types of behaviour for peak intensity were identified. (i) No clear trends between the degrees of ripeness, but relatively repeatable data between replicates. E.g. 1-hexanol showed the highest intensity in *Tipica* for half-ripe, whereas in *Catuai* the half-ripe beans had the lowest intensity of the three degrees of ripeness – Fig. 4a. (ii) Very different intensities between the two varieties, with possible but small differences dependent on the degree of ripeness. For example, higher intensity for 2,6-dimethyl pyridine was observed in *Catuai* beans (Fig. 4b), while the intensity for 2,3-butanediol was higher in *Tipica* beans (Fig. 4c). (iii) Furfural (Fig. 4d) was differentiating between the ripeness levels of *Catuai*, whereas no differentiation was observed for *Tipica*. The unripe and half-ripe *Catuai* samples had higher furfural signals than the corresponding *Tipica* samples. In contrast, furfural signal was much lower in the ripe *Catuai* beans than in the ripe *Tipica* beans.

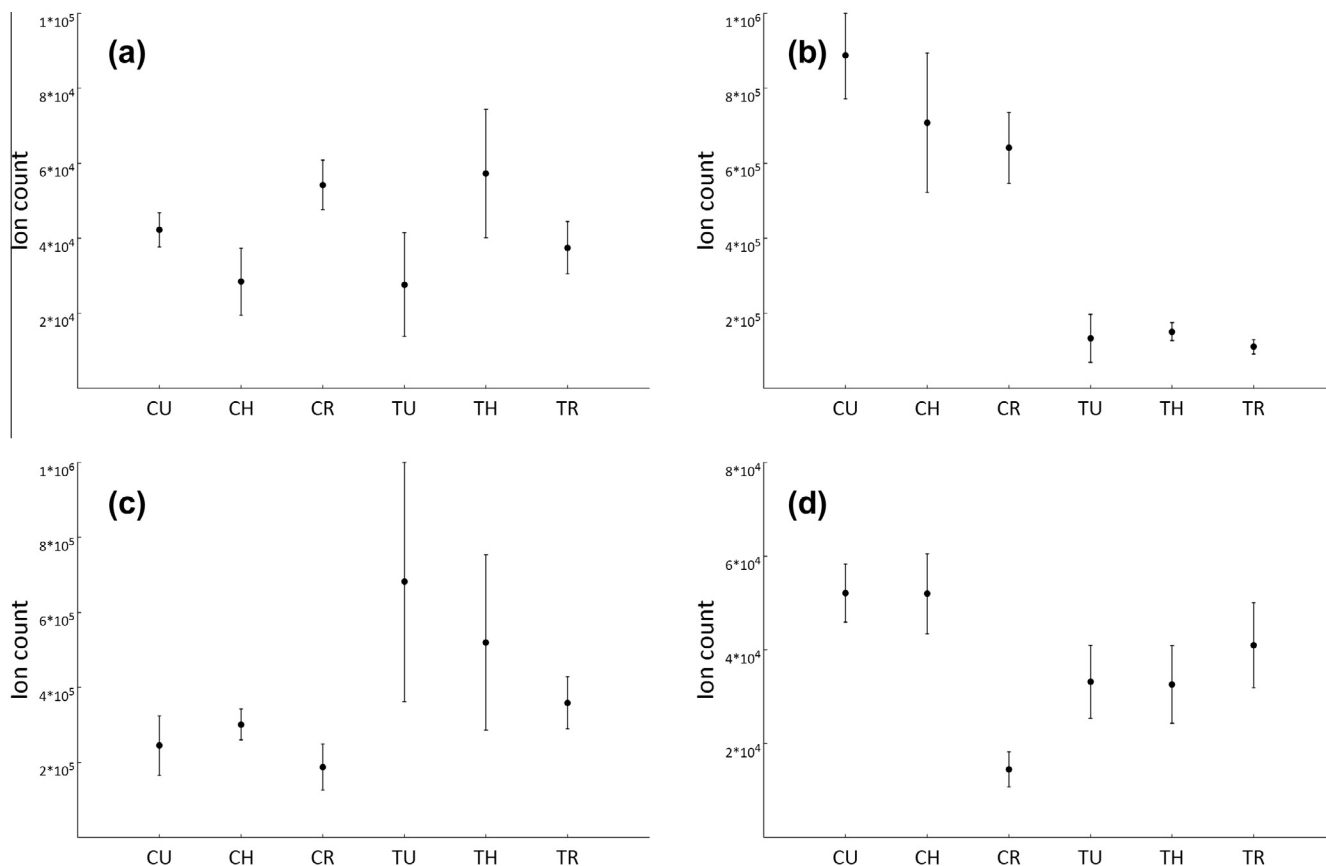
### 3.4. Statistical analysis

In order to extract differences between samples across the two varieties and three degrees of ripeness, the various datasets were

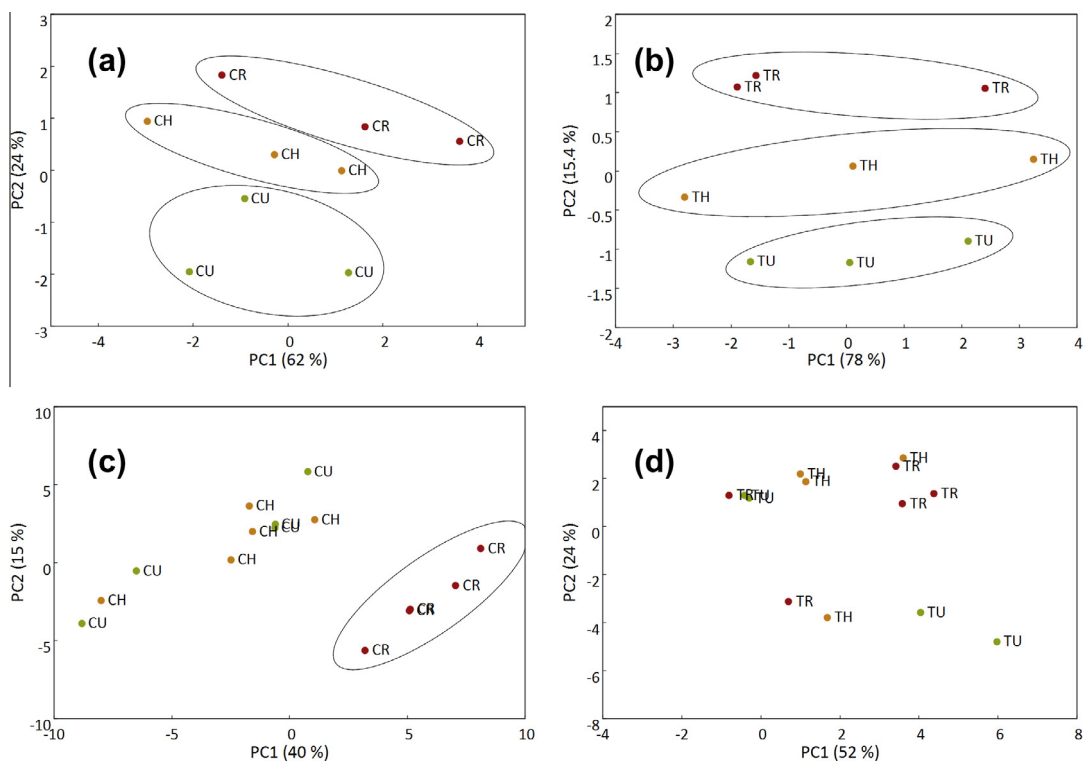
analysed by principal component analysis (PCA). A statistical analysis with PCA of the RP-HPLC data showed very good separation in the degree of ripeness especially along PC2 (24% loadings) for both the *Catuai* (Fig. 5a) and the *Tipica* (Fig. 5b) samples. The loadings showed that there is an increase in 3-CQA content and decreases in 5-CQA and di-CQAs for both coffee varieties, with increasing degree of ripeness. The decrease in the content of some of the CGAs compares well to data published in the literature. An indication of a drop in the di-CQAs (Jham et al., 2001) as degree of ripeness increased (immature, ripe, overripe) has been reported. A similar observation was reported in another study (Koshiro et al., 2007) examining unprocessed beans, where the authors reported a decrease in di-CQA and 5-CQA and an increase in 3-CQA with ripening. While their study covered a much larger range of degree of ripeness, the trends are consistent with our observations over a narrower range of degree of ripeness.

Analysis of the headspace volatile profile using PCA showed a separation between ripe *Catuai* sample and the unripe and half-ripe ones (Fig. 5c), but no separation based on the degree of ripeness was seen for the *Tipica* samples (Fig. 5d). Based on the loadings, the separation between the ripe samples was caused by an increase in hexanal, pentanoic acid and hexanoic acid, and a decrease in furfural signals.

HS volatile profiling of whole green coffee beans is a quick and simple method and has successfully been applied for the detection of defective beans (Toci & Farah, 2008, 2014), however, in our work, this approach did not prove to be robust enough to distinguish between the degrees of ripeness. Further studies into the optimisation of SPME parameters are needed to improve reproducibility and check for the usefulness of the method for this application.



**Fig. 4.** Peak areas of selected volatile compounds in the headspace above whole green coffee beans: (a) 1-hexanol, (b) 2,6-dimethyl pyridine, (c) 2,3-butanediol, (d) furfural for *Catuai* (CU – *Catuai* unripe, CH – *Catuai* half-ripe, CR – *Catuai* ripe) and *Tipica* (TU – *Tipica* unripe, TH – *Tipica* half-ripe, TR – *Tipica* ripe) samples. Error bars show standard deviation,  $n = 5$ .



**Fig. 5.** PCA plots of RP-HPLC data for *Catuai* (a) and *Tipica* samples (b) and HS SPME GC/MS data for *Catuai* (c) and *Tipica* samples (d). CU – *Catuai* unripe; CH – *Catuai* half-ripe; CR – *Catuai* ripe; TU – *Tipica* unripe; TH – *Tipica* half-ripe; TR – *Tipica* ripe.

#### 4. Conclusion

This study has focused on the possibility of finding differences between green coffee beans that were harvested at different degrees of ripeness. A set of chromatographic methods was developed and optimised to analyse methanol and water green coffee extracts and to measure the headspace composition above whole green coffee beans. Differences between both coffee varieties were larger than those between the different degrees of ripeness. The best discrimination between the degrees of ripeness was obtained using RP-HPLC and very good differentiation between samples was achieved using PCA. The separation between the different degrees of ripeness can be attributed to an increase in 3-CQA and a decrease in 5-CQA and di-CQAs. The total area of the HMW fraction at 280 nm in the HPSEC analysis showed clear differences between both the degrees of ripeness and the two coffee varieties. In addition, by analysing the composition of the headspace above green coffee beans, clear differences between both varieties were observed, but only the ripe *Catuai* sample could be differentiated in terms of ripeness using PCA. Hence, this study indicates that non-volatiles are more suited to differentiate between different degrees of ripeness of green coffee beans, while headspace profiles are more appropriate for determining differences between the two varieties examined.

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