Enhancing Robusta Coffee aroma by modifying flavour precursors in the green coffee bean

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Highlights:

1. Varying levels of sugars were used to pre-treat Robusta green beans.
2. Treatment increased the similarity of Robusta to Arabica.
3. The optimum level of sugar treatment was Robusta soaked in 15F solution.
4. For coffee aroma the blending ratio can be increased from 20% to 80% Robusta.
5. The aroma of treated Robusta coffee was more stable than Arabica.

Abstract

This study attempted to improve Robusta sensory properties by modifying the beans chemical composition. Building on our previous work, which modified bean pH through acid pre-treatment, a model system was developed where, sugar solutions (glucose, fructose, sucrose) were used to pre-treat Robusta coffee beans with the aim to modify the concentration/availability/location of these aroma precursors. Beans were then dried to equal water activity, subjected to equal roast intensity and ground to comparable particle size distributions. The treatment significantly impacted aroma generation during roasting leading to an altered level of pyrazines, furans, ketones, organic acid and heterocyclic nitrogen-containing compounds ($p < 0.05$). The optimum treatment was 15 g/100g fructose. 80% treated Robusta could be blended with Arabica in coffee brew without significant aroma differences being perceived when compared to 100% Arabica brew. Furthermore the aroma of the fructose treated Robusta was more stable than Arabica over six weeks accelerated shelflife storage.

1 Introduction

Being a popular beverage worldwide, coffee demand and consumption have increased significantly over recent years. The International Coffee Organization estimated that two billion cups are consumed every day and of which the fastest growing segment is for premium coffee, therefore there is an urgent need to improve beverage quality without increasing cost (International Coffee Organization, 2016). Cup quality depends on various factors therefore scientists have found it challenging to improve coffee quality due to the complexity within the bean and the processing.

Green coffee bean chemical composition plays an important role in aroma formation during the roasting process (Fisk, Kettle, Hofmeister, Virdie, & Kenny, 2012). The Maillard reaction is the major pathway of aroma formation in coffee, amino acids and reducing sugars react to form nitrogenous heterocycles and brown melanoidins (Illy & Viani, 2005). This non-enzymatic browning produces hundreds of volatile compounds, and contributes to a number of sensory attributes of coffee (Lersch, 2012). Controlling the precursors (sugars, amino acids) and the process will therefore enable control over the aroma generation and the final flavour of the coffee (Wong, Abdul Aziz, & Mohamed, 2008).

The two main cultivated species of coffee are Arabica (*Coffeea Arabica* L.) and Robusta (*Coffeea canephora* P.) (Illy & Viani, 2005). Previous studies have showed that Arabica has a sweet, caramel roast aroma whilst Robusta has an earthy, spicy roast aroma (Blank, Sen, & Grosch, 1991). Sucrose is considered important for the development of the organoleptic qualities of coffee and Robusta has significantly less (2.7% dry weight) compared to the 6% (dwb) that is found in Arabica (Illy & Viani, 2005). The higher sucrose content results in an enhanced aroma formation for Arabica (Farah, 2012). In Argentina, Spain and Singapore, there is a special type of roasted coffee called *Torrefacto* which it is produced by roasting whole beans with sucrose or glucose (maximum proportion is around 15% of added

50 sugar during roasting process) (Wrigley, 1988). The sugar added in this treatment is proposed not to
51 increase the sweetness of the coffee brew but to protect the beans from oxidation by forming a thin sugar
52 film on the surface and to speed up the Maillard reaction (Wrigley, 1988). This procedure has also been
53 demonstrated to mask the poor quality of low grade beans, especially Robusta (Lersch, 2012).

54 Our previous study involved the treatment of green coffee beans with a solution containing varying
55 concentration of acetic acid for 2 h at 20 ˚C, with the aim to change the acidity of bean prior to roasting
56 therefore diverting the kinetics of certain reaction pathways that occur during aroma formation during
57 roasting, this treatment reduced the aroma differences between Arabica and Robusta and enabled a higher
58 blending ratio (Liu, Yang, Linforth, Fisk, & Yang, 2018). We are building on this previous work, that
59 highlighted the importance of the local microchemistry (pH) on aroma generation, and offer an
60 alternative, more targeted method to alter the concentration/availability/location of sugar precursors for
61 Maillard chemistry and caramelisation reactions that occur during roasting. Instead of modifying the
62 local solvent micro-chemistry (pH), the objective of this study is therefore to develop a model system
63 that allows us for the first time to individually modify the green bean chemical precursors (sucrose,
64 glucose and fructose), and individually evaluate their impact on the coffee aroma generation and to show
65 that modification of flavour precursors could be used to increase the aroma similarity between Arabica
66 and Robusta coffee and further to understand the impact on aroma stability over shelf life.

67 Compared with Torrefacto process, instead of adding sugar during the roasting process, our study
68 modified the flavour precursors content in the green beans prior to roasting. Green Robusta beans were
69 pre-soaked in solutions of both reducing sugars (glucose and fructose) and a non-reducing sugar (sucrose)
70 at a range of concentrations (0 – 15g/100g) under 2 bar pressure and a rotation of 1 rpm using a steam
71 retort to modify the green bean sugar content. Aroma analysis was carried out after coffee roasting by

Gas chromatography mass spectrometry (GC-MS) with headspace solid phase micro extraction (SPME). Sensory analysis in aroma was performed to determine the largest proportion of Robusta or treated Robusta that could be blended with Arabica without any perceived sensory differences and accelerated shelf life testing performed to explain the impact on aroma stability during storage.

2 Materials and methods

2.1 Coffee Samples

Robusta samples were single-origin washed green beans from Vietnam. High grade Arabica coffee samples (Type AA: cupping 93/100) were sourced from Aberdares, Mount Kenya. They were both supplied by Edgehill coffee UK. Green coffee beans were positioned into a Modulyo Freeze Dryer 1311-03/08 JM (Edwards, Crawley, UK) at −40 ˚C for 72 h until they achieved a humidity less than 5% before treatment. Freeze dried Robusta green beans were soaked with varying concentrations of individual sugar solution (glucose, fructose and sucrose) (Sigma-Aldrich, Poole, UK) with concentrations of 0, 3, 6, 9, 12, and 15 g/100g for 30 min at 100 ˚C with 2 bar pressure and a rotation of 1 rpm using a steam retort with four replicates each. Control samples were treated with water only. Moisture content after treatment was controlled as detailed in our previous work (Liu, Yang, Linforth, Fisk, & Yang, 2018), in brief treated coffee was dried naturally and placed into a salt chamber with saturated salt solution for two weeks (moisture content 11.5% ± 0.5%). Measurement of water loss over time was conducted by weighing the coffee samples at every step.

All coffee samples (4 replicates each) were roasted in the same batch using a 10 sample tray convection oven (Mono Equipment, Swansea, UK) for 20 min at 200 °C and, after cooling by air, were ground using a coffee grinder (KG 49, Delonghi, Australia). Ground coffee was stored in a sealed aluminium bag at -80 ˚C after sieving (sieve size 710 μm Endecotts, Essex, UK).

2.2 Coffee Samples for Storage Test

Coffee was stored at 5, 25, and 35 ˚C in a laboratory oven (Sanyo, Loughborough, UK). The moisture content of all samples before storage were measured less than 2%. Samples were removed after 2, 4 and

6 weeks and stored at -80 ˚C (4 replicate samples). Control samples were stored from the start of the trial at -80 ˚C. For instrumental analysis, all samples were analysed together at the end of the storage test in a randomised order.

2.3 Gas Chromatograph Mass Spectrometry (GC-MS)

1.5 g of samples were placed into GC headspace vials (20 mL, 22.5 mm × 75.5 mm, Sigma-Aldrich, UK) (four replicates). 3-Heptanone was used as internal standard (15 µL, 0.01% 3-Heptanone (Sigma, Saint Louis, USA) in methanol (Laboratory reagent grade, Fisher Scientific, UK)) to calibrate for any instrument drift.

Aroma sampling conditions were chosen according to Liu, Yang, Linforth, Fisk, & Yang, (2018), where optimal conditions for pre-equilibrium time and temperature, extraction and injection are reported. In brief, analysis was conducted using a trace 1300 series Gas Chromatography coupled with the Single-Quadrupole Mass Spectrometer (Thermo Fisher Scientific, Hemel Hempstead, UK). Samples were incubated with shaking at 40 ˚C for 5 min. A 50/30 µm DVB/CAR/PDMS SPME Fibre (Supelco, Sigma Aldrich, UK) was used to extract volatile compounds from the headspace of each samples. The SPME fibre was extracted for 5 min then thermally desorbed for 2 min at 200 ˚C, splitless mode, constant carrier pressure of 18 psi, and then separated by GC-MS.

The column was a 30 m length ZB-WAX capillary column (0.25 mm internal diameter and 1.00 µm film thickness, Phenomenex, Macclesfield, UK). The conditions were as follows: 40 ˚C for 5 min, ramped to 180 ˚C at 3 ˚C /min, and then ramped to 240 ˚C at 8 ˚C /min, held for 2 min. Full scan mode was used in a mass range of m/z 20 to 300.

Volatile compounds were identified by comparison of each mass spectrum with either the spectra from standard compounds or with spectra in reference libraries (NIST/EPA/NIH Mass Spectral Library, version 2.0, Faircom Corporation, U.S.). The relative abundant of volatiles was calculated from GC peak areas, by comparison with the peak area of the internal standard.All samples were analysed in one run in randomised order.

2.4 Measurement for Physical Properties

Colour was determined for four replicates with a Hunter Lab (ColourQuest XE, HunterLab, US) to produce lightness (L), a value, and b value. Positive a and b represent red and yellow, negative a and b represent green and blue respectively (Hunter Lab, 2008). The conditions of the experiment were as follows: standard illumination: D65, colorimetric normal observer angle: 10°, ASTM E308 RSIN Mode, LAV, 1.00 Port, UV Nominal. The readings were made by CIELAB system. The Hunter Lab was standardized by using the light trap standard (serial no. CQX2614) and diagnostic tile (serial no. CQX2614). Coffee powders (1g) were put into cuvettes (SARSTEDT AG & Co. D-51588) and directly placed to the measurement aperture to test L, a and b value with three positions selected at random. The total colour difference (∆E), ∆E also can be calculated by equation and represents the difference between the treated samples and the Arabica control.

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

2.5 Sugar Analysis by Liquid Chromatography-Mass Spectrometry

Coffee powder (0.1 g) was positioned in a 50 mL centrifuge tube with 15 mL of boiling water and vortexed for 5 min. Samples then were centrifuged at 1600 g for 10 min at ambient temperature. After centrifugation, the liquid phase was transferred into a new glass vial. The above processes was repeated

three times. The mixture was cooled to room temperature and then filtered using a syringe filter (0.45 µm, 40 hydrophilic nylon syringe filter, Millipore Corporation). The final extract was diluted with methanol (MeOH) (1:1) prior to Liquid chromatography-mass spectrometry (LC/MS) analysis (the method was modified from Caporaso, Whitworth, Grebby, & Fisk, (2018) and Perrone, Donangelo, & Farah, (2008).

The LC equipment (1100 Series, Agilent) consisted of a degasser (G1322A, Agilent), a pump (G1312A, Agilent), an auto-sampler (G1313A, Agilent). This LC system was interfaced with a Quattro Ultima mass spectrometer (Micromass, UK Ltd.) fitted with an electrospray ion source. The Luna 5u NH2 100A column (250 ×3.20 mm, 5 µm, Phenomenex) was used to separate sucrose, glucose and fructose at room temperature. Chromatographic separation was carried with an isocratic elution mobile phase of 80% acetonitrile. The flow rate was set at 0.7 mL/min, the volume injected was 5 µL.

Peaks were determined by comparing retention times to those of standard compounds. Calibration curves were made of sucrose, glucose and fructose standards (Sigma Aldrich®). Standards were prepared at concentration of 1, 2.5, 5, 7.5, and 10 mg/mL in 50:50 MeOH:H_2O. The respective peak areas were used for the quantification.

2.6 Sensory Evaluation

Robusta samples treated by soaking in 15 g/100g fructose (15F) were selected to be tested in the sensory study. The coffee brew for sensory evaluation were freshly brewed in a cafetière just before the test start to avoid any flavour loss and oxidation. According manufacturers' instruction, 54 g of coffee was weighed and add in the 8-cup capacity cafetière (Argos, Stafford, UK). 860 mL boiling water was then poured into the cafetière with 5 times stir. The coffee were then wait for 3 min before depressing the

plunger. Brewed coffee (10 mL) was then poured into amber glass vessels and cooled down to room temperature (20 ± 2 ˚C) for sniffing test.

This study was approved by School of Bioscience Ethic Committee at the University of Nottingham (SBREC160138A), a small incentive was provided to participants. All sensory tests were conducted under northern hemisphere lighting at the Sensory Science Centre of the University of Nottingham in the individual sensory booths. Ninety-eight volunteers were recruited from students and staff at University of Nottingham, all participants have signed informed consent. Participants were invited for one session which lasted approximately 30 min, in the session, a total of 7 triangle tests were carried out. The objective of the sensory test was to determine the similarity between non-treated Robusta and Arabica and the blended Arabica with Robusta (treated or control). In previous studies we have shown that participants can perceive when a minimum of 40% of Robusta is blended with Arabica (Liu, Yang, Linforth, Fisk, & Yang, 2018). Therefore, in this experiment, a blending ratio of 20% and 40% Robusta with Arabica were compared with 100% Arabica to confirm this finding. For fructose-treated Robusta, samples with 20%, 40%, 60% and 80% blending with Arabica were used to compare with 100% Arabica.

For each triangle test, three samples were given to the volunteers, and they were instructed to smell the samples from left to right and select the odd one. A two minute break was given between triangles tests. No other prior knowledge or training was given to the assessors. A randomised sampling order was used between and within each triangle test.

2.7 Statistical Analysis

Experiments were carried out in quadruplicate. Data is presented as a mean value with standard deviation and samples were compared by analysis of variance (ANOVA) using samples as the fixed effect and

followed by Tukey's HSD post-hoc test, $p < 0.05$ was regarded as significant. All statistical analyses were conducted using either IBM® SPSS® Statistics version 21.0.0 or Excel XLSTAT (Version 2015.5.01.23373). All sensory data was collected and analysed using Compusense Cloud (Compusense, Ontario, Canada). Number of responses was compared to the critical tables in BS EN ISO 4120: 2007 (α=0.05 for difference testing; $\alpha = 0.2$, $\beta = 0.05$, pD = 30% for similarity testing).

3. Results and discussion

3.1 Impact of Treatment on Sugar Content and Bean Colour after Roasting

The sugar content in the green coffee beans and the colour of the roasted coffee beans are presented in Table 1. Non-treated Robusta had significantly lower concentrations of sucrose when compared with Arabica (respectively: 3.20 g/100g ± 0.38; 6.20 g/100g ± 0.10) ($p < 0.05$). There was no significant difference in the glucose concentration between Arabica and non-treated Robusta ($p \geq 0.05$). However, the fructose concentration in the non-treated Robusta (0.76 g/100g ± 0.20) was significantly higher than Arabica (0.13 g/100g ± 0.06).

To accelerate the diffusion of sucrose, glucose and fructose into the coffee beans, pre-soaking was carried out at 2 bar pressure. A rotation of 1 rpm was used to create even distribution of the treatment solution. The process control (water treated Robusta) was significantly lower in sucrose, glucose and fructose content when compared with the non-treated Robusta. This is due to the nature of the treatment process as, sucrose, glucose and fructose are water soluble and can be leached out into the process water during the treatment.

Increasing the sugar concentration in the treatment solution increased the sugar content in the treated green beans (Table 1). At the highest treatment level, Robusta samples were treated by soaking in 15 g/100g of individual sugars (fructose, glucose, and sucrose), which are represented as 15F, 15G and 15S accordingly. There was 4.98 g/100g sucrose in the 15S treated green beans; 7.39 g/100g glucose in the 15G treated green bean; 7.35 g/100g 15F in the fructose treated green bean. At the highest sucrose treatment level the treated Robusta coffee still had a lower sucrose concentration (4.98 g/100g) than Arabica (6.20 g/100g). There was a significant increase in glucose and fructose concentrations between

206 the glucose and fructose treated Robusta samples compared with the Arabica sample (Table 1). It should
207 be noted that less sucrose was detected in the sucrose treated samples than glucose or fructose in their
208 treated samples. Sucrose is a disaccharide with the molecular weight 342 g/mol and may penetrate the
209 sample matrix less readily than monosaccharides such as glucose (180 g/mol) and fructose (180 g/mol).

210 Colour analysis of the coffee bean samples showed significant differences in L, a, b ($p < 0.05$) between
211 Arabica beans and the non-treated Robusta. ΔE was used to determine the overall distance between two
212 colours. According to the previous study, ΔE of 3.0 is the minimum colour difference that human eyes
213 can detect (depends on the hue) (Martínez-Cervera, Salvador, Muguerza, Moulay, & Fiszman, 2011).
214 Clear differences were seen between the Arabica and the non-treated Robusta with a total colour
215 difference ΔE of 7.48 (Table 1). This is the greatest colour difference between the Arabica and all coffee
216 samples. At 15S treatment, 12G and 15G treatment and 9F, 12F and 15F treatment, total colour
217 differences were lower than 3, and were the least colour difference when compared with Arabica. As a
218 result, it can be seen that sugar pre-treatment reduced the colour difference between Arabica and Robusta
219 after roasting.

220 Increasing the levels of flavour precursors (sucrose, glucose and fructose) in the Robusta beans did alter
221 the colour of the beans making the treated coffee more similar to that of the Arabica bean. The colour
222 formation is mainly due to the Maillard reaction (Bastos, 2012) and sugar caramelization processes,
223 which can occur simultaneously, hence it is hard to separate the two reactions (Wong, Abdul Aziz, &
224 Mohamed, 2008). It should be noted that the reducing sugars (glucose and fructose) had a greater impact
225 on the colour change than the non-reducing sugar sucrose suggests that both Maillard reaction and
226 caramelization are of importance. Ganesan and Benjakul did a similar study on the basis of glucose
227 treatment on pidan white (pickled duck eggs). They hypothesised and proved that adding Maillard

chemistry precursors (glucose) could improve brown colour development principally through accelerating the Maillard reaction (Ganesan, Benjakul, & Baharin, 2014), which consistent with our result in table 1.

3.2 Determination of the Volatile Compounds in Coffee after Treatment

Thirty-four volatile compounds were identified in all coffee samples, they was screened and selected as compounds that have previously been shown to be key aroma compounds with sensory significance in coffee. These aroma compounds are shown in table 2 and include 5 furans, 2 organic acids, 5 heterocyclic compounds (N containing), 4 sulphur-containing compounds, 2 aldehydes, 3 ketones and 9 pyrazines, 1 ether, 1 alcohol and 2 phenolic compounds. Their linear retention index, identification method and related odour description are illustrated in Table 2.

3.3 Summary of All Coffee Samples via Volatile Chemistry

Principal component analysis (PCA) was used to illustrate the variation in the level of the 34 volatiles compounds formed during the roasting process (Figure 1). The first principal component (PC1) represents 63.9% of the variance in the whole dataset and was negatively correlated with pyrazines and phenolic compounds and positively correlated with furans, ketones, aldehydes, ether, alcohol and acids on the right. The second principal component (PC2) represents 18.6% of the variance and has a positive correlation with pyrroles and negative correlation with sulphur-containing compounds. The non-treated Robusta sample had greater levels of pyrazines and phenolic compounds (left with triangle mark). While Arabica have a positive correlation with acids, furans, ketones and aldehydes (right with triangle mark). The main categories of compounds found at a higher proportion in Arabica were furans, acids, aldehydes and pyridines, which literature suggests are related to the aroma of roasted sweet caramel (Petisca, Pérez-

Palacios, Farah, Pinho, & Ferreira, 2013). Robusta on the other hand is known to have a spicy burnt earthy odour due to higher concentrations of pyrazines and derivatives (Kerler, 2010), which is concordant with our results in the Figure 1. Increasing the levels of flavour precursors (sucrose, fructose and glucose)moved the aroma profile from left to right, closer to Arabica. The 15F treated coffees (square marked in the figure 1) was the closest to the Arabica samples.

The extent of the change in aroma profile was more marked for the reducing sugars (glucose and fructose) when compared to the non-reducing sugar (sucrose) suggestions that whilst caramelisation may be important, Maillard chemistry is the major drives factor in the change in aroma profile and is critically important for binding the gap between Arabica and Robusta.

3.4 Aroma Chemistry

The aroma profile for Arabica, treated and non-treated Robusta sample is illustrated in Figure 2, where the level of 34 key volatile compounds in treated and non-treated Robusta coffee are normalised by their respective concentrations in Arabica coffee (100%). Significant differences were shown in all 34 key aroma compounds between Arabica and Robusta (Figure 2 (a)). Robusta coffee had 2 to 4 times higher concentration of all pyrazines, pyrroles, phenolic compounds and 4-Methylthiazole when compare with Arabica coffee. However, for the rest of the volatile compounds, such as furans, ketones, aldehydes, and acids, non- treated Robusta coffee had up to 8 times lower concentration than Arabica coffee.

As shown in figure 2 (b), the aroma profile for the process control Robusta sample indicated significant differences ($p < 0.001$) in 32 volatile compounds compared to Arabica apart from pyrrole and disufide dimethyl. These include a significantly greater level of pyrazines, phenolic compounds and 4-methylthiazole and lower levels of compounds such as furans, ketones, acids and aldehydes. Similar to

non-treated Robusta, the process control Robusta had a similar pattern but the differences were smaller. These included a significantly decreased levels of compounds such as pyrazines, furans, aldehydes, ketones and pyrroles. This change can be explained by the leaching of water soluble precursors during treatment process as shown in table 1. Volatiles such as furfural, 2-methylfuran have been reported as sugar degradation products that can be affected in this way (Flament, 2002). In addition, an alteration to the bean density (from 0.75 g/mL to 0.62 g/mL) could also alter the thermal reaction pathways during aroma formation. High density beans are more resistant to absorption of heat and takes a longer time to roast (Pittia, Dalla Rosa, & Lerici, 2001). Applying steam and pressure to the beans may open up bean pores and could modify the density of the green coffee beans. As a result, treated beans could have a lower density and be less resistant to heat.

Figure 2 (c) indicated the aroma profile between Arabica and 15 F treated Robusta. There were no significant differences in the concentration of 16 compounds (including all pyrazines, aldehydes, 2, 5-dimethylfuran, 4-methylthiazole, *4*-vinylguaiacol, 1-ethylpyrrole and 2, 5-dimethylpyrrole) between Arabica and 15F treated Robusta. Although most furans, ketones and organic acids were still lower in the 15F treated Robusta coffee compared with the Arabica, all furans, ketones and organic acids indicated a significant increase in 15F treated Robusta (2-3 fold) when compare with non-treated Robusta and processing controlled Robusta, which made it closer to Arabica's profile.

Figure 2 (d) indicated the aroma profile between Arabica and 15G treated Robusta. There were no significant differences in 6 compounds (including 2, 5-dimethylpyrazine, 2-ethyl-5-methylpyrazine, methylpyrazine, pyrazine, 1-ethylpyrrole, and 2, 5-dimethylfuran) between Arabica coffee and 15G treated Robusta coffee. Some pyrazines (2, 5-Dimethylpyrazine, 2-ethyl-5-methylpyrazine, methyl pyrazine, pyrazine) indicated a significant decrease in 15G treated Robusta (60% - 100%) compared with

292 non-treated Robusta (Figure 2 (a)). The concentration of 1-ethylpyrrole and 2, 5-dimetylfuran increased
293 around 30% to 50% respectively in the 15G treated Robusta when compared with the non-treated one.

294 Figure 2 (e) shows the aroma profile between Arabica and 15S treated Robusta. There were no significant
295 difference in the concentration of 7 compounds (2, 5-dimethylpyrazine, 2-ethyl-5-methylpyrazine,
296 methylpyrazine, pyrazine, 1-ethylpyrrole, 2, 5-dimethylfuran and furfural) between Arabica coffee and
297 15S treated Robusta coffee. Both glucose treated Robusta (15G) and sucrose treated Robusta (15S) had
298 a similar pattern, apart from the relative concentration of furfural, which showed a significant increase
299 in 15S treated Robusta (26%) compared with 15G treated Robusta sample.

300 The significant rise in the ketone, furan and acid compounds in the sugar treated Robusta may due to the
301 formation of those compounds through carbohydrate pyrolysis and sugar degradation (Flament, 2002).
302 Research has revealed that sugar decomposition enhances the volatilization and formation of formic acid,
303 acetic acid and lactic acid in the initial stages of roasting (Yeretzian, Jordan, Badoud, & Lindinger, 2002).
304 In the later stages, during roasting at high temperature, furaneol and hydroxymethylfurfural are generated
305 via sugar caramelization. However, aroma formation is more likely through the Maillard route than
306 caramelization due to lower activation energy in the presence of reactive nitrogen species (amino acids)
307 (Hodge, 1953; Yeretzian, Jordan, Badoud, & Lindinger, 2002). The formations of these furans is thought
308 to be greatly dependent on the sugar content (Nie et al, 2013). The sugar treatment level could therefore
309 affect the formation of furans. Pyrazine is known to be predominant in Robusta and is formed by amino
310 acids and reducing sugars following the Maillard reaction (Ehiling et al 2005). Koehler, and Odell 1970,
311 discovered that increasing (3 fold) the amounts of sugar added could decrease the concentration of
312 pyrazines generated, and the assumption was that excess sugar affected the reactant ratio hence

decreasing pyrazine levels. That could also be the reason for the lower pyrazine levels observed in sugar treated Robusta.

Pyrroles and pyridines were significantly decreased (around 2 fold) in the sugar treated Robusta (Figure 2 (c), (d), (e)). These two groups of compounds are formed as a result of the thermal degradation of Amadori intermediates. The intermediate products can either cyclize to form these nitrogenous heterocyclic compounds, or go to a different route where cleavage and formation of rearranged sugars occur. Due to the rearranged sugars comprising of the intact chain of the starting sugar and the original amine that was liberated, less or different volatile aroma compound were created (Jousse, Jongen, Agterof, Russell, & Braat, 2002). Moreover, pyrroles and pyridines have also been reported as pyrolysis products of trigonelline (Flament, 2002). The reduced pyrroles and pyridines relative concentration may be therefore due to the trigonelline leaching out during the pre-treatment process, which is confirmed by the process control (Figure 2 (b)).

Of the three different sugars used to treat Robusta samples (15F, 15S and 15G), 15F treated Robusta sample was found to be the optimum treatment conditions with the most compounds showing no significant difference compare with Arabica. It indicated that the formation of the volatile compounds can be affected by the types of sugar involved in the Maillard reaction and caramelization during the roasting process, as also reported by Brands & Van Boekel, 2001. Reducing sugar both glucose and fructose (monosaccharides) were more reactive than the non-reducing sugar sucrose (disaccharides) (Van Boekel & Brands, 2005).

For monosaccharides, ketoses such as fructose give rise to the corresponding Heyns compound, whilst the Aldoses such as glucose give rise to the Amadori intermediate compounds (Brands & Van Boekel, 2001). There are conflicting reports in the literature regarding the issue of reactivity of sugars, several

studies (Spark, 1969; Baxter, 1995) support that glucose is more reactive, while other researches claim that fructose is more reactive (Kato, Yamamoto, & Fujimaki, 1969; Mauron, 1981; Suarez, Etlinger, Maturana, & Weitman, 1995; Walton, McPherson, & Shilton, 1989). Further studies indicated that the relative reaction rates vary for both glucose and fructose depending on the reaction conditions (Brands & Van Boekel, 2001; Laroque, Inisan, Berger, Vouland, Dufossé, & Guérard, 2008; Rewicki, Kersten, Helak, Nittka, & Tressl, 2005).

In our study, 15F treated Robusta generated more furans, ketones, aldehydes and acetic acid compared with 15G treated Robusta, which agreed with the study on the flavour precursors in the Maillard reaction done by Kraehenbuehl et al. 2010. On the other hand, formation of pyrazines significantly decreased in 15F treated Robusta compared with 15G treated Robusta. No significant difference in pyrazines can be observed in the 15F treated Robusta compared with Arabica. As discussed above, only 15F treated Robusta samples were used for the sensory evaluation.

3.5 Influence of Accelerated Shelf-life Storage on the Volatile Compounds

The relative change (percentage) in aroma of the three coffee samples stored for six weeks at 35 ˚C is shown in figure 3. The relative aroma difference during storage was normalised to 100% of its original level in each coffee. The use of relative abundance in figure 3 was used to avoid different starting points for Arabica, Robusta and treated Robusta coffee before storage as these two varieties might contain different amounts of the volatile compounds after roasting.

For Arabica, all compounds significantly decreased over the storage period between 25% - 60% ($p < 0.05$). The only exception was acids that increased around two fold over the six weeks' time. The concentrations of total pyrroles, pyrazines, aldehydes, furans reduced significantly during six week

storage at 35 ˚C in Arabica, non-treated Robusta and 15F treated Robusta. Non-treated Robusta, treated Robusta and Arabica all showed no significant difference in the ketones after six weeks stored at 35 ˚C when compared with the control.

The aroma of 15F treated Robusta was more stable during 6 weeks storage compared with Arabica, as most of the volatiles in Arabica coffee showed a greater loss over storage when compared to the treated Robusta. The only exception was that 15F treated Robusta generated 35% more acids (include acetic acid and propanoic acid) compared with Arabica during the six weeks stored. The formation of acetic acid can be due to degradation of small to medium chained carbohydrates such as glucose, sucrose and fructose (Illy & Viani, 2005). The higher fructose content may result in a greater acid release in the roasted coffee (Farah, 2012; Rewicki, Kersten, Helak, Nittka, & Tressl, 2005). Moreover, previous studies on staling and rancidity in coffee concluded that the volatile compounds (such as furfural and acetaldehyde) can be oxidised to the corresponding volatile acids during coffee storage period (Elder, 1937). 15F treated Robusta coffee generated around 25% more furfural compared with Arabica (Figure 2 (c)). Therefore, higher volatile acids formation during coffee storage could also be explained by the oxidation of aroma constituents. Whilst the difference in stability of aroma compounds in the Arabica compared to the Robusta and treated Robusta cannot be clearly explained, it may be due to the present of different levels of micro nutrients, different volatiles and different bean chemistry. However, it is clear that the aroma of Robusta and treated Robusta were more stable. This was especially evident for pyrazines, aldehydes and furans.

3.6 Sensory evaluation

Fructose treated Robusta coffee (15F) was blended with up to 80% Arabica coffee and compared with the Arabica control to identify the maximum blend ratio without a perceive aroma difference. The results

for the numbers of correct responses in a sensory triangle test evaluation of brewed coffee are shown in Table 3. According to ISO4120:2007, samples were classed as being similar to Arabica if the number of correct responses was less than 40 out of 98.

In agreement with Liu, Yang, Linforth, Fisk, & Yang (2018), participants could not tell a difference between Arabica and Arabica containing 20% Robusta blend, but once the blending ratio increased to 40% Robusta, participants could tell that the aroma was significantly different from the 100% Arabica sample. Interestingly, when comparing Arabica with 15F treated Robusta blended with Arabica, participants could not discriminate between the aroma of the two samples, no matter the percentage of the blending (from 20% to 80% blends). The sensory evaluation results are consistent with the volatile analysis which showed that the 15F treated samples were the most similar to Arabica, and enable therefore on an aroma basis an increase in blending ratio from 20% Robusta 80% Arabica to 80% treated Robusta 20% Arabica .

4. Conclusions

In conclusion, this project has successfully developed a model system for the evaluation of flavour precursors in green beans and proposed how modifying green bean carbohydrate profile can result in an enhanced aroma profile where the aroma of Robusta coffee is more similar to Arabica. Analytical results indicated that the inclusion of fructose resulted in the most similar aroma profile to Arabica. Sensory test results validated this finding, which proved that 15F treated Robusta had a similar perceived aroma as Arabica. The maximum permissible blending proportion of Robusta increased from 20% for the non-treated Robusta coffee to 80% for the 15F treated Robusta coffee. It is clear from these findings that modification of the aroma precursors (especially fructose addition) changes the roasted coffee aroma profile and enables a higher Robusta blending ratio. Furthermore, the aroma stability of the treated Robusta significantly increased.

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