1    **Enhancing Robusta Coffee aroma by modifying flavour precursors in the green**

2    **coffee bean**

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8    **Keywords:**

9    Coffee processing; Green bean pre-treatment; Sugar; Shelf-life; Sensory analysis; Aroma chemistry

10    **Highlights:**

11    1. Varying levels of sugars were used to pre-treat Robusta green beans.

12    2. Treatment increased the similarity of Robusta to Arabica.

13    3. The optimum level of sugar treatment was Robusta soaked in 15F solution.

14    4. For coffee aroma the blending ratio can be increased from 20% to 80% Robusta.

15    5. The aroma of treated Robusta coffee was more stable than Arabica.

16 **Abstract**

17 This study attempted to improve Robusta sensory properties by modifying the beans chemical

18 composition. Building on our previous work, which modified bean pH through acid pre-treatment, a

19 model system was developed where, sugar solutions (glucose, fructose, sucrose) were used to pre-treat

20 Robusta coffee beans with the aim to modify the concentration/availability/location of these aroma

21 precursors. Beans were then dried to equal water activity, subjected to equal roast intensity and ground

22 to comparable particle size distributions. The treatment significantly impacted aroma generation during

23 roasting leading to an altered level of pyrazines, furans, ketones, organic acid and heterocyclic nitrogen-

24 containing compounds ($p < 0.05$). The optimum treatment was 15 g/100g fructose. 80% treated Robusta

25 could be blended with Arabica in coffee brew without significant aroma differences being perceived

26 when compared to 100% Arabica brew. Furthermore the aroma of the fructose treated Robusta was more

27 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

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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

72    Gas chromatography mass spectrometry (GC-MS) with headspace solid phase micro extraction (SPME).

73    Sensory analysis in aroma was performed to determine the largest proportion of Robusta or treated

74    Robusta that could be blended with Arabica without any perceived sensory differences and accelerated

75    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

97 6 weeks and stored at -80 ˚C (4 replicate samples). Control samples were stored from the start of the trial

98 at -80 ˚C. For instrumental analysis, all samples were analysed together at the end of the storage test in

99 a randomised order.

100 *2.3 Gas Chromatograph Mass Spectrometry (GC-MS)*

101 1.5 g of samples were placed into GC headspace vials (20 mL, 22.5 mm × 75.5 mm, Sigma-Aldrich, UK)

102 (four replicates). 3-Heptanone was used as internal standard (15 µL, 0.01% 3-Heptanone (Sigma, Saint

103 Louis, USA) in methanol (Laboratory reagent grade, Fisher Scientific, UK)) to calibrate for any

104 instrument drift.

105 Aroma sampling conditions were chosen according to Liu, Yang, Linforth, Fisk, & Yang, (2018), where

106 optimal conditions for pre-equilibrium time and temperature, extraction and injection are reported. In

107 brief, analysis was conducted using a trace 1300 series Gas Chromatography coupled with the Single-

108 Quadrupole Mass Spectrometer (Thermo Fisher Scientific, Hemel Hempstead, UK). Samples were

109 incubated with shaking at 40 ˚C for 5 min. A 50/30 µm DVB/CAR/PDMS SPME Fibre (Supelco, Sigma

110 Aldrich, UK) was used to extract volatile compounds from the headspace of each samples. The SPME

111 fibre was extracted for 5 min then thermally desorbed for 2 min at 200 ˚C, splitless mode, constant carrier

112 pressure of 18 psi, and then separated by GC-MS.

113 The column was a 30 m length ZB-WAX capillary column ( 0.25 mm internal diameter and 1.00 µm film

114 thickness, Phenomenex, Macclesfield, UK). The conditions were as follows: 40 ˚C for 5 min, ramped to

115 180 ˚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

116 a mass range of m/z 20 to 300.

117 Volatile compounds were identified by comparison of each mass spectrum with either the spectra from

118 standard compounds or with spectra in reference libraries (NIST/EPA/NIH Mass Spectral Library,

119 version 2.0, Faircom Corporation, U.S.). The relative abundant of volatiles was calculated from GC peak

120 areas, by comparison with the peak area of the internal standard.All samples were analysed in one run in

121 randomised order.

122 *2.4 Measurement for Physical Properties*

123 Colour was determined for four replicates with a Hunter Lab (ColourQuest XE, HunterLab, US) to

124 produce lightness (L), a value, and b value. Positive a and b represent red and yellow, negative a and b

125 represent green and blue respectively (Hunter Lab, 2008). The conditions of the experiment were as

126 follows: standard illumination: D65, colorimetric normal observer angle: 10°, ASTM E308 RSIN Mode,

127 LAV, 1.00 Port, UV Nominal. The readings were made by CIELAB system. The Hunter Lab was

128 standardized by using the light trap standard (serial no. CQX2614) and diagnostic tile (serial no.

129 CQX2614). Coffee powders (1g) were put into cuvettes (SARSTEDT AG & Co. D-51588) and directly

130 placed to the measurement aperture to test L, a and b value with three positions selected at random. The

131 total colour difference ($\Delta$E), $\Delta$E also can be calculated by equation and represents the difference between

132 the treated samples and the Arabica control.

133 $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$

134 *2.5 Sugar Analysis by Liquid Chromatography-Mass Spectrometry*

135 Coffee powder (0.1 g) was positioned in a 50 mL centrifuge tube with 15 mL of boiling water and

136 vortexed for 5 min. Samples then were centrifuged at 1600 g for 10 min at ambient temperature. After

137 centrifugation, the liquid phase was transferred into a new glass vial. The above processes was repeated

138   three times. The mixture was cooled to room temperature and then filtered using a syringe filter (0.45

139   µm, 40 hydrophilic nylon syringe filter, Millipore Corporation). The final extract was diluted with

140   methanol (MeOH) (1:1) prior to Liquid chromatography-mass spectrometry (LC/MS) analysis (the

141   method was modified from Caporaso, Whitworth, Grebby, & Fisk, (2018) and Perrone, Donangelo, &

142   Farah, (2008).

143   The LC equipment (1100 Series, Agilent) consisted of a degasser (G1322A, Agilent), a pump (G1312A,

144   Agilent), an auto-sampler (G1313A, Agilent). This LC system was interfaced with a Quattro Ultima mass

145   spectrometer (Micromass, UK Ltd.) fitted with an electrospray ion source. The Luna 5u NH2 100A

146   column (250 ×3.20 mm, 5 µm, Phenomenex) was used to separate sucrose, glucose and fructose at room

147   temperature. Chromatographic separation was carried with an isocratic elution mobile phase of 80%

148   acetonitrile. The flow rate was set at 0.7 mL/min, the volume injected was 5 µL.

149   Peaks were determined by comparing retention times to those of standard compounds. Calibration curves

150   were made of sucrose, glucose and fructose standards (Sigma Aldrich®). Standards were prepared at

151   concentration of 1, 2.5, 5, 7.5, and 10 mg/mL in 50:50 MeOH:$H_2O$. The respective peak areas were used

152   for the quantification.

153   *2.6 Sensory Evaluation*

154   Robusta samples treated by soaking in 15 g/100g fructose (15F) were selected to be tested in the sensory

155   study. The coffee brew for sensory evaluation were freshly brewed in a cafetière just before the test start

156   to avoid any flavour loss and oxidation. According manufacturers' instruction, 54 g of coffee was

157   weighed and add in the 8-cup capacity cafetière (Argos, Stafford, UK). 860 mL boiling water was then

158   poured into the cafetière with 5 times stir. The coffee were then wait for 3 min before depressing the

159    plunger. Brewed coffee (10 mL) was then poured into amber glass vessels and cooled down to room

160    temperature (20 ± 2 ˚C) for sniffing test.

161    This study was approved by School of Bioscience Ethic Committee at the University of Nottingham

162    (SBREC160138A), a small incentive was provided to participants. All sensory tests were conducted

163    under northern hemisphere lighting at the Sensory Science Centre of the University of Nottingham in the

164    individual sensory booths. Ninety-eight volunteers were recruited from students and staff at University

165    of Nottingham, all participants have signed informed consent. Participants were invited for one session

166    which lasted approximately 30 min, in the session, a total of 7 triangle tests were carried out. The

167    objective of the sensory test was to determine the similarity between non-treated Robusta and Arabica

168    and the blended Arabica with Robusta (treated or control). In previous studies we have shown that

169    participants can perceive when a minimum of 40% of Robusta is blended with Arabica (Liu, Yang,

170    Linforth, Fisk, & Yang, 2018). Therefore, in this experiment, a blending ratio of 20% and 40% Robusta

171    with Arabica were compared with 100% Arabica to confirm this finding. For fructose-treated Robusta,

172    samples with 20%, 40%, 60% and 80% blending with Arabica were used to compare with 100% Arabica.

173    For each triangle test, three samples were given to the volunteers, and they were instructed to smell the

174    samples from left to right and select the odd one. A two minute break was given between triangles tests.

175    No other prior knowledge or training was given to the assessors. A randomised sampling order was used

176    between and within each triangle test.

177    *2.7 Statistical Analysis*

178    Experiments were carried out in quadruplicate. Data is presented as a mean value with standard deviation

179    and samples were compared by analysis of variance (ANOVA) using samples as the fixed effect and

180 followed by Tukey's HSD post-hoc test, $p < 0.05$ was regarded as significant. All statistical analyses

181 were conducted using either IBM® SPSS® Statistics version 21.0.0 or Excel XLSTAT (Version

182 2015.5.01.23373). All sensory data was collected and analysed using Compusense Cloud (Compusense,

183 Ontario, Canada). Number of responses was compared to the critical tables in BS EN ISO 4120: 2007

184 ($\alpha$=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 $\pm$ 0.38; 6.20 g/100g $\pm$ 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 $\pm$ 0.20) was significantly higher than Arabica (0.13 g/100g $\pm$ 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

228 chemistry precursors (glucose) could improve brown colour development principally through

229 accelerating the Maillard reaction (Ganesan, Benjakul, & Baharin, 2014), which consistent with our

230 result in table 1.

*3.2 Determination of the Volatile Compounds in Coffee after Treatment*

232 Thirty-four volatile compounds were identified in all coffee samples, they was screened and selected as

233 compounds that have previously been shown to be key aroma compounds with sensory significance in

234 coffee. These aroma compounds are shown in table 2 and include 5 furans, 2 organic acids, 5 heterocyclic

235 compounds (N containing), 4 sulphur-containing compounds, 2 aldehydes, 3 ketones and 9 pyrazines, 1

236 ether, 1 alcohol and 2 phenolic compounds. Their linear retention index, identification method and related

237 odour description are illustrated in Table 2.

*3.3 Summary of All Coffee Samples via Volatile Chemistry*

239 Principal component analysis (PCA) was used to illustrate the variation in the level of the 34 volatiles

240 compounds formed during the roasting process (Figure 1). The first principal component (PC1)

241 represents 63.9% of the variance in the whole dataset and was negatively correlated with pyrazines and

242 phenolic compounds and positively correlated with furans, ketones, aldehydes, ether, alcohol and acids

243 on the right. The second principal component (PC2) represents 18.6% of the variance and has a positive

244 correlation with pyrroles and negative correlation with sulphur-containing compounds. The non-treated

245 Robusta sample had greater levels of pyrazines and phenolic compounds (left with triangle mark). While

246 Arabica have a positive correlation with acids, furans, ketones and aldehydes (right with triangle mark).

247 The main categories of compounds found at a higher proportion in Arabica were furans, acids, aldehydes

248 and pyridines, which literature suggests are related to the aroma of roasted sweet caramel (Petisca, Pérez-

249    Palacios, Farah, Pinho, & Ferreira, 2013). Robusta on the other hand is known to have a spicy burnt

250    earthy odour due to higher concentrations of pyrazines and derivatives (Kerler, 2010), which is

251    concordant with our results in the Figure 1. Increasing the levels of flavour precursors (sucrose, fructose

252    and glucose)moved the aroma profile from left to right, closer to Arabica. The 15F treated coffees (square

253    marked in the figure 1) was the closest to the Arabica samples.

254    The extent of the change in aroma profile was more marked for the reducing sugars (glucose and fructose)

255    when compared to the non-reducing sugar (sucrose) suggestions that whilst caramelisation may be

256    important, Maillard chemistry is the major drives factor in the change in aroma profile and is critically

257    important for binding the gap between Arabica and Robusta.

258    *3.4 Aroma Chemistry*

259    The aroma profile for Arabica, treated and non-treated Robusta sample is illustrated in Figure 2, where

260    the level of 34 key volatile compounds in treated and non-treated Robusta coffee are normalised by their

261    respective concentrations in Arabica coffee (100%). Significant differences were shown in all 34 key

262    aroma compounds between Arabica and Robusta (Figure 2 (a)). Robusta coffee had 2 to 4 times higher

263    concentration of all pyrazines, pyrroles, phenolic compounds and 4-Methylthiazole when compare with

264    Arabica coffee. However, for the rest of the volatile compounds, such as furans, ketones, aldehydes, and

265    acids, non- treated Robusta coffee had up to 8 times lower concentration than Arabica coffee.

266    As shown in figure 2 (b), the aroma profile for the process control Robusta sample indicated significant

267    differences ($p < 0.001$) in 32 volatile compounds compared to Arabica apart from pyrrole and disufide

268    dimethyl. These include a significantly greater level of pyrazines, phenolic compounds and 4-

269    methylthiazole and lower levels of compounds such as furans, ketones, acids and aldehydes. Similar to

270  non-treated Robusta, the process control Robusta had a similar pattern but the differences were smaller.

271  These included a significantly decreased levels of compounds such as pyrazines, furans, aldehydes,

272  ketones and pyrroles. This change can be explained by the leaching of water soluble precursors during

273  treatment process as shown in table 1. Volatiles such as furfural, 2-methylfuran have been reported as

274  sugar degradation products that can be affected in this way (Flament, 2002). In addition, an alteration to

275  the bean density (from 0.75 g/mL to 0.62 g/mL) could also alter the thermal reaction pathways during

276  aroma formation. High density beans are more resistant to absorption of heat and takes a longer time to

277  roast (Pittia, Dalla Rosa, & Lerici, 2001). Applying steam and pressure to the beans may open up bean

278  pores and could modify the density of the green coffee beans. As a result, treated beans could have a

279  lower density and be less resistant to heat.

280  Figure 2 (c) indicated the aroma profile between Arabica and 15 F treated Robusta. There were no

281  significant differences in the concentration of 16 compounds (including all pyrazines, aldehydes, 2, 5-

282  dimethylfuran, 4-methylthiazole, *4*-vinylguaiacol, 1-ethylpyrrole and 2, 5-dimethylpyrrole) between

283  Arabica and 15F treated Robusta. Although most furans, ketones and organic acids were still lower in

284  the 15F treated Robusta coffee compared with the Arabica, all furans, ketones and organic acids indicated

285  a significant increase in 15F treated Robusta (2-3 fold) when compare with non-treated Robusta and

286  processing controlled Robusta, which made it closer to Arabica's profile.

287  Figure 2 (d) indicated the aroma profile between Arabica and 15G treated Robusta. There were no

288  significant differences in 6 compounds (including 2, 5-dimethylpyrazine, 2-ethyl-5-methylpyrazine,

289  methylpyrazine, pyrazine, 1-ethylpyrrole, and 2, 5-dimethylfuran) between Arabica coffee and 15G

290  treated Robusta coffee. Some pyrazines (2, 5-Dimethylpyrazine, 2-ethyl-5-methylpyrazine, methyl

291  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

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

314    treated Robusta.

315    Pyrroles and pyridines were significantly decreased (around 2 fold) in the sugar treated Robusta (Figure

316    2 (c), (d), (e)). These two groups of compounds are formed as a result of the thermal degradation of

317    Amadori intermediates. The intermediate products can either cyclize to form these nitrogenous

318    heterocyclic compounds, or go to a different route where cleavage and formation of rearranged sugars

319    occur. Due to the rearranged sugars comprising of the intact chain of the starting sugar and the original

320    amine that was liberated, less or different volatile aroma compound were created (Jousse, Jongen,

321    Agterof, Russell, & Braat, 2002). Moreover, pyrroles and pyridines have also been reported as pyrolysis

322    products of trigonelline (Flament, 2002). The reduced pyrroles and pyridines relative concentration may

323    be therefore due to the trigonelline leaching out during the pre-treatment process, which is confirmed by

324    the process control (Figure 2 (b)).

325    Of the three different sugars used to treat Robusta samples (15F, 15S and 15G), 15F treated Robusta

326    sample was found to be the optimum treatment conditions with the most compounds showing no

327    significant difference compare with Arabica. It indicated that the formation of the volatile compounds

328    can be affected by the types of sugar involved in the Maillard reaction and caramelization during the

329    roasting process, as also reported by Brands & Van Boekel, 2001. Reducing sugar both glucose and

330    fructose (monosaccharides) were more reactive than the non-reducing sugar sucrose (disaccharides) (Van

331    Boekel & Brands, 2005).

332    For monosaccharides, ketoses such as fructose give rise to the corresponding Heyns compound, whilst

333    the Aldoses such as glucose give rise to the Amadori intermediate compounds (Brands & Van Boekel,

334    2001). There are conflicting reports in the literature regarding the issue of reactivity of sugars, several

335 studies (Spark, 1969; Baxter, 1995) support that glucose is more reactive, while other researches claim

336 that fructose is more reactive (Kato, Yamamoto, & Fujimaki, 1969; Mauron, 1981; Suarez, Etlinger,

337 Maturana, & Weitman, 1995; Walton, McPherson, & Shilton, 1989). Further studies indicated that the

338 relative reaction rates vary for both glucose and fructose depending on the reaction conditions (Brands

339 & Van Boekel, 2001; Laroque, Inisan, Berger, Vouland, Dufossé, & Guérard, 2008; Rewicki, Kersten,

340 Helak, Nittka, & Tressl, 2005).

341 In our study, 15F treated Robusta generated more furans, ketones, aldehydes and acetic acid compared

342 with 15G treated Robusta, which agreed with the study on the flavour precursors in the Maillard reaction

343 done by Kraehenbuehl et al. 2010. On the other hand, formation of pyrazines significantly decreased in

344 15F treated Robusta compared with 15G treated Robusta. No significant difference in pyrazines can be

345 observed in the 15F treated Robusta compared with Arabica. As discussed above, only 15F treated

346 Robusta samples were used for the sensory evaluation.

347 *3.5 Influence of Accelerated Shelf-life Storage on the Volatile Compounds*

348 The relative change (percentage) in aroma of the three coffee samples stored for six weeks at 35 ˚C is

349 shown in figure 3. The relative aroma difference during storage was normalised to 100% of its original

350 level in each coffee. The use of relative abundance in figure 3 was used to avoid different starting points

351 for Arabica, Robusta and treated Robusta coffee before storage as these two varieties might contain

352 different amounts of the volatile compounds after roasting.

353 For Arabica, all compounds significantly decreased over the storage period between 25% - 60% ($p <$

354 0.05). The only exception was acids that increased around two fold over the six weeks' time. The

355 concentrations of total pyrroles, pyrazines, aldehydes, furans reduced significantly during six week

356    storage at 35 ˚C in Arabica, non-treated Robusta and 15F treated Robusta. Non-treated Robusta, treated

357    Robusta and Arabica all showed no significant difference in the ketones after six weeks stored at 35 ˚C

358    when compared with the control.

359    The aroma of 15F treated Robusta was more stable during 6 weeks storage compared with Arabica, as

360    most of the volatiles in Arabica coffee showed a greater loss over storage when compared to the treated

361    Robusta. The only exception was that 15F treated Robusta generated 35% more acids (include acetic acid

362    and propanoic acid) compared with Arabica during the six weeks stored. The formation of acetic acid

363    can be due to degradation of small to medium chained carbohydrates such as glucose, sucrose and

364    fructose (Illy & Viani, 2005). The higher fructose content may result in a greater acid release in the

365    roasted coffee (Farah, 2012; Rewicki, Kersten, Helak, Nittka, & Tressl, 2005). Moreover, previous

366    studies on staling and rancidity in coffee concluded that the volatile compounds (such as furfural and

367    acetaldehyde) can be oxidised to the corresponding volatile acids during coffee storage period (Elder,

368    1937). 15F treated Robusta coffee generated around 25% more furfural compared with Arabica (Figure

369    2 (c)). Therefore, higher volatile acids formation during coffee storage could also be explained by the

370    oxidation of aroma constituents. Whilst the difference in stability of aroma compounds in the Arabica

371    compared to the Robusta and treated Robusta cannot be clearly explained, it may be due to the present

372    of different levels of micro nutrients, different volatiles and different bean chemistry. However, it is clear

373    that the aroma of Robusta and treated Robusta were more stable. This was especially evident for

374    pyrazines, aldehydes and furans.

375    *3.6 Sensory evaluation*

376    Fructose treated Robusta coffee (15F)  was blended with up to 80% Arabica coffee and compared with

377    the Arabica control to identify the maximum blend ratio without a perceive aroma difference. The results

378    for the numbers of correct responses in a sensory triangle test evaluation of brewed coffee are shown in

379    Table 3. According to ISO4120:2007, samples were classed as being similar to Arabica if the number of

380    correct responses was less than 40 out of 98.

381    In agreement with Liu, Yang, Linforth, Fisk, & Yang (2018), participants could not tell a difference

382    between Arabica and Arabica containing 20% Robusta blend, but once the blending ratio increased to

383    40% Robusta, participants could tell that the aroma was significantly different from the 100% Arabica

384    sample. Interestingly, when comparing Arabica with 15F treated Robusta blended with Arabica,

385    participants could not discriminate between the aroma of the two samples, no matter the percentage of

386    the blending (from 20% to 80% blends). The sensory evaluation results are consistent with the volatile

387    analysis which showed that the 15F treated samples were the most similar to Arabica, and enable

388    therefore on an aroma basis an increase in blending ratio from 20% Robusta 80% Arabica to 80% treated

389    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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