Impact of water-soluble precursors leaching from green beans on aroma generation during coffee roasting

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Abstract
In this investigation green Robusta coffee beans were pre-soaked with different time-temperature profiles before roasting in normal conditions and grinding to a standardized particle size. Aroma profile of roasted coffee beans and water soluble precursors such as sucrose and total protein content from soaking water were examined by using Solid Phase Micro Extraction -Gas Chromatograph Mass Spectrometry and Liquid chromatography–mass spectrometry and BCA Protein Assay Kit respectively. A significant impact of soaking time-temperature profile was observed on the yield of water-soluble precursors in the soaking water. The loss of these precursors significantly decreased aroma formation during roasting. The results also suggested that water-soluble precursors could modify the quality of Robusta coffee.

Introduction
Coffee species such as Arabica and Robusta are most common coffee varieties in the world, which account for 61% and 38% of the coffee production worldwide. Arabica, perceived as a smooth, and rich flavour is usually more desirable than Robusta, which is often described as having a muddy odour. Robusta coffee beans are often blended with Arabica coffee beans to create specific aroma profiles, enhance cream formation or reduce cost, but the maximum that can be included is often limited due to the loss of aroma quality [5].

Aroma formation in coffee is directly related to the chemical composition of the green coffee beans and typical coffee aromas are developed during the roasting process due to complex reactions such as, Maillard reactions, Strecker degradation, thermal degradation and oxidation [2]. A number of studies have improved the quality of Robusta coffee by passing the green Robusta beans through steam to remove substances such as 2-methylisoborneol, which is responsible for the muddy odour [1]. However, during this process important water soluble precursors such as sucrose and protein are leached into water, hence compromising the flavour generation potential of the roasted coffee. The amino acids and sugar are considered to be the main precursors in the aroma generation and colour formation during coffee roasting [3]. Therefore, the objective of this study was to investigate how much water-soluble precursors are lost during pre-soaking of green coffee beans and its impact on aroma generation during coffee roasting.

Experimental
Coffee preparation
Coffee beans were purchased from Edgehill coffee, Warwick, United Kingdom, where both Robusta beans (Vietnam) and Arabica beans (Kenya) are single-origin washed beans. Robusta green beans were soaked in water solution at different time (2, 4, 6, 8, 10 and 12 h) and temperatures (20, 40, 60, 80 and 100 °C), four replicates each. Soaked Robusta green beans and non-treated Robusta green were placed into a desiccator with saturated sodium nitrate solution (relative humidity 65.5%) at room temperature.
(20±2 °C) for 20 d to control the moisture content (around 11.5%). Determination of the water changes during soaking and coffee roasting was carried out by weighting the coffee sample at every step. Soaked Robusta green beans and non-treated Robusta green beans were roasted in a convection oven (Mono Equipment, Swansea, UK) at 200 °C for 20 min. Roasted samples were ground with an electronic coffee grinder (KG 49, Delonghi, Australia) then passed through a metal sieve size 710 um (Endecotts, Essex, UK) and stored in the freezer at -80°C prior to analysis.

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

The ground coffee (1.5 g) was transferred into glass vials (20 ml), four replicates for GC-MS analysis. An internal standard was prepared by adding 10 μL 3-heptanone (Sigma, Saint Louis, USA) into 10 ml methanol (Laboratory reagent grade, Fisher Scientific, UK). 2 μL of internal standard was added into each coffee sample and kept for 1 h equilibrium prior to GC analysis. All analytical samples were randomised for GC-MS analysis. A trace 1300 series Gas Chromatograph coupled with the Single-Quadrupole Mass Spectrometer (Thermo Fisher Scientific, Hemel Hemptead, UK) was used for analysis of volatile compounds. Samples were incubated at 40 °C for 5 min with shaking. A 50/30 μm DVB/CAR/PDMS SPME Fibre (Supelco, Sigma Aldrich, UK) was used to extract volatile compounds from the sample headspace (extraction for 5 min then desorption for 2 min). The injector temperature was set at 200 °C in splitless mode (constant carrier pressure was at 18 psi). Separation was carried out on a ZB-WAX Capillary GC Column (length 30 m, inner diameter 0.25 mm, film thickness 1 μm; Phenomenex Inc., Macclesfield, UK). Column temperature was held initially at 40 °C for 5 min, increased by 3 °C/min to 180 °C, then 8 °C/min to 240 °C and held for 2 min. Full scan mode was used to detect the volatile compounds (mass range from m/z 20 to 300).

**BCA protein assay kit and Liquid Chromatography-Mass Spectrometry (LC-MS)**

Pierce TM BCA protein assay kit (23225/23227, Thermo Scientific) was used to measure the total protein content for both green beans and soaking water. Liquid Chromatography-Mass Spectrometry (LC-MS) was used to measure the sucrose content for both green beans and soaking water. The LCMS analysis was performed following standard protocol described in Perrone et al, 2008. All results were analysed by Design-Expert version 7.0.0 and Microsoft excel 2010 using samples as the fixed effect and a Tukey’s HSD post-hoc test. Principal Component Analysis (PCA) was performed by Excel XLSTAT Version 2015.5.01.23373.

**Results and discussion**

**Water-soluble precursors**

In figure 1, protein content showed a significant decrease with increased soaking temperature (p < 0.05). Similarly, a significant decrease in the sucrose content was observed in soaked Robusta green beans at 20 °C for 12 h when compared with non-soaked Robusta green beans. However, there were no significant differences between the soaked green beans at 20 °C and 40 °C for 12 h. Significant decrease in the sucrose content showed in the soaking temperature at 60 °C, 80 °C and 100 °C for 12 h. A significant decrease in the sucrose content was observed with at 60, 80 and 100 °C soaking temperature for 12 h.
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Figure 1: Sucrose and protein content in the non-soaked and soaked Robusta green beans at different soaking temperature at constant soaking time (12 h). The error bars are standard derivation.

Figure 2 showed that the soaking time also play an important role on the sucrose and protein content. In summary, a significant impact of soaking temperature and time on the water soluble precursors from green coffee beans was observed, this can significantly impact the aroma profile of roasted coffee beans ($p < 0.001$). In addition, increase soaking temperature results in higher loss in protein (from 8.3\% to 3.3\%) and sucrose (from 3.1\% to 1.6\%) content (Figure 1) when compare with increase soaking time the protein loss from 7.04\% to 3.3\% and sucrose from 2.6\% to 1.6\% (Figure 2).

Figure 2: Sucrose and protein content in the non-soaked and soaked Robusta green beans at constant soaking temperature 100˚C for different soaking time. The error bars are standard derivation

Aroma

A range of volatile compounds were observed with roasted Robusta coffee beans with different functional groups such as 2 organic acids, 1 alcohol, 2 aldehydes, 3 ketones, 2 furans, and 5 heterocyclic compounds (N containing). All aroma compounds showed a significant decrease in their content with increasing soaking time and temperature ($p < 0.05$). These volatile compounds are associated with sensory odour description such as malty, nutty, grassy, sour, burnt, and smoky [3].

Principal component analysis (PCA) was used to illustrate the variation between the 15 aroma compounds across the 10 soaked Robusta samples (including different time and temperature) and 1 non-soaked Robusta sample (Figure 3). PCA results indicated that both soaking time and temperature have a significant effect on aroma generation during the coffee roasting. The first principal component (PC1) accounted for 75.51\% of the
variance in the whole dataset and showed separation between the soaked Robusta (left) and non-soaked Robusta samples (right). The second principal component (PC2) accounted for 17.37% of the variance in the dataset and discriminated the difference between increasing soaking time (top) and soaking temperature (bottom). Sample soaked at 20 °C and 40 °C for 12 h, showed more closed to the furfural, acetic acid, 2-methylfuran, 2, 3-butanedione, 2-furanmethanol and 2, 3-pentanedione content as compared to the samples soaked at a higher temperature (60 °C, 80 °C and 100 °C). Non-soaked beans have a significantly higher concentration of all these volatile compounds (p < 0.001) as compared to soaked green beans. This change can be explained by the leaching of sucrose during soaking process at higher temperature as shown in Figure 1. Volatiles such as furfural, acetic acid, 2-methylfuran, 2, 3-butanedione, 2-furanmethanol and 2, 3-pentanedione have been reported as sugar degradation products [3]. Therefore, in conclusion the reduction sucrose content in the soaked green beans has significantly affected aroma formation during the roasting process.

Figure 3: Principle component analysis (Bi-plot) of the volatiles compounds associated with soaked and non-soaked Robusta coffee analysed by GC-MS.

References