COFFEE FLAVOUR MODULATION – REINFORCING THE FORMATION OF KEY ODORANTS WHILE MITIGATING UNDESIRABLE COMPOUNDS

L. POISSON¹, J. Kerler¹, Ch. Milo¹, F. Schmalzried¹,², T. Davidek¹, and I. Blank¹

¹ Nestlé Product Technology Centre Orbe, Nestec LTD., CH-1350 Orbe, Switzerland
² University of Hohenheim, Institute for Food Chemistry, Stuttgart, Germany

Abstract

A combination of biomimetic in-bean experiments and spiking of green coffee beans with potential precursors was implemented to study formation pathways of several key odorants like 2-furfurylthiol (FFT) and alkylpyrazines during coffee roasting. Both labelled and unlabelled precursor molecules were used and the target analytes in the roasted coffee samples were analysed in terms of their isotope labelling pattern and their abundance. The biomimetic experiments elucidated that FFT is very likely not generated via 2-furaldehyde, which is in contrast to what was found in model studies. In addition, the incorporation of the arabinose C5-skeleton into FFT could not be confirmed, and our study showed that smaller arabinose fragments are integrated into the FFT molecule. In the second series of experiments, proposed pathways for the formation of alkylpyrazines were confirmed and the role of amino acids (e.g. alanine) was underpinned. In conclusion, the results of the biomimetic in-bean experiments emphasised the potential of this methodology for the verification of formation pathways in complex food systems like coffee. Furthermore, it represents a tool for the evaluation of options to modulate the aroma profile of roast and ground coffee.

Introduction

The formation of important coffee aroma compounds that belong to the group of thiols and pyrazines has been extensively studied in model systems under dry heating conditions (1-3). This has recently been extended to undesirable compounds such as furan (4, 5). In arabinose/cysteine model experiments, Tressl et al. (1) showed that 2-furfurylthiol (FFT) is formed via 3-deoxypentosone and 2-furaldehyde while maintaining the intact carbon chain. Further experiments with polysaccharides isolated from green coffee and roasted in the presence of cysteine performed by Grosch (2) provided evidence that arabinogalactans are key precursors of FFT.

Amrani-Hemaimi et al. (3) showed in different model studies that C6 and C5 sugars (e.g. fructose, glucose or arabinose) are potential precursors for alkylpyrazines whose degradation compounds form key intermediates (i.e. α-amino-oxo compounds) through the Strecker reaction. In addition, alanine and glycine play a key role as they are integrated into the side chain of the alkylpyrazine molecule (3).

However, as it has been recently demonstrated for the formation of furan from ascorbic acid, the conclusions from model systems have to be taken with caution and cannot simply be extrapolated to complex food products (4). Hence, to study the importance of precursors for the formation of key aroma compounds during coffee roasting under real conditions, the so-called biomimetic in-bean experiments were developed. The idea of this approach is to make use of the green coffee beans as
Expression of Multidisciplinary Flavour Science

'mini reactor' for model reactions, which allows a more realistic evaluation of potential precursors and provides very useful insight into formation pathways. The method involves the extraction of the green coffee by hot water, followed by the replacement of the solubilised fraction by a compounded equivalent. This biomimetic recombined extract (BRE) can be selectively omitted or fortified in particular precursors or, for mechanistic studies, labelled precursors may be incorporated. In addition, spiking of untreated green coffee beans with precursors or precursor groups represent another straightforward tool to studying the modulation of coffee flavour.

Coffee flavour modulation is an even more challenging task when aiming at optimisation of flavour generation and the mitigation of undesirable molecules such as furan, as both odorants and process contaminants were found to be formed from common precursors (4, 5). Therefore, our study aimed at verifying formation pathways of several key odorants such as FFT and alkylpyrazines in the coffee bean. In parallel, furan was monitored in order to identify strategies for its mitigation.

Experimental

**Biomimetic in-bean experiments.** Hot water extraction of green coffee beans. Green coffee beans were extracted with hot water as reported in the literature (6) using some modifications. 70 kg of green Arabica coffee beans (Colombia) were extracted consequently four times with demineralised water at 95 °C for a total of 2 h to obtain the water soluble substances. The extracts were collected and concentrated to a total solid content of approximately 27 %. After that, both the exhausted beans and the aqueous natural green bean extract were freeze-dried and stored at -40 °C until use.

**Incorporation of biomimetic recombined extracts (BRE).** The reconstituted green coffee extract (BRE, based on analytical results of the water soluble green coffee composition) was dissolved in demineralised water at 80 °C. 50 g of water were used for 125 g exhausted beans (EB) in order to guarantee a complete incorporation of the model solution into the coffee beans. The pH value of the compounded water soluble fraction was adjusted to 5.5 (corresponding to the pH of the natural extract) with a 16.5% w/w solution of KOH, and water exhausted green coffee beans were soaked with the BRE at 50 °C for at least 5 h. During soaking, the beans were gently stirred using a Rotavapor.

**Omission experiments.** BRE extracts with omission of potential precursors or precursor groups were reincorporated in the exhausted coffee beans as described before. The compounded extracts were omitted in all free sugars as well as all free amino acids.

**Mechanistic studies.** D-[U-13C5]-arabinose (0.9 g) was added to the compounded model extract, which was omitted in all sugars, and incorporated into the water extracted, exhausted green beans. Additionally, green coffee beans were spiked with L-[3-13C]-alanine (0.48 g/150 g beans).

**Spiking of precursor compounds.** For the flavour modulation experiments, untreated green beans were fortified with equimolar amounts of different sugars such as glucose, arabinose (approximately 4 g of each sugar), or sucrose (about 50% of the natural amount in coffee), or amino acids like cysteine (0.45 g/150 g beans) or alanine (0.48 g/150 g beans). Each of the precursors was dissolved in 60 g demineralised water and green coffee beans (150 g) were soaked with the prepared solution for 2 h at 50 °C and for 2 h at room temperature. All treated green coffee samples were freeze-dried and then roasted at same conditions on a “Signum” rotating fluidized bed roaster (Neuhaus-Neotec, Germany) for 380 s at 236 °C.
Instrumental analysis. Quantification by Solid Phase Micro Extraction (SPME) combined with GC/MS. R&G coffee was suspended in hot water to obtain a slurry and after cooling spiked with defined quantities of labelled isotopes of the analytes. The prepared coffee suspensions were equilibrated (60 min, 20 °C) in the sealed vials and the aroma compounds were extracted from the headspace (10 min, 40 °C) using SPME (2 cm fibre coated with PDMS/DVB/Carboxen; Supelco). Aroma compounds were thermally desorbed in the injector port of the GC at 240 °C coupled to a mass spectrometer (Thermo DSQ). Separation of compounds was achieved on a polar silica thin-film capillary (Phenomenex ZB-Wax, 60 m × 0.25 mm; film thickness, 0.25 μm). Absolute concentrations are expressed as relative amounts compared to the reference set at 100 % (biomimetic recombined beans and green coffee).

Results and Discussion

2-Furfurythiol (FFT). Milo et al. (6) reconfirmed previous work on models that FFT is mainly formed from water non-soluble precursors. In their biomimetic studies increased amounts of FFT were found in roasted coffee from water-extracted, exhausted beans. Indeed, the omission of all water soluble sugars in the biomimetic recombined green coffee resulted in significantly increased amounts of FFT, whereas 2-furaldehyde content was highly suppressed to less than 40% as compared to a fully reconstituted R&G coffee (BRE; see Figure 1A). Spiking experiments also did not show a relationship between the formation of FFT and 2-furaldehyde, as fortification of green beans with sucrose (50% of natural content) increased 2-furaldehyde amounts up to 160%, whereas concentrations of FFT considerably decreased (Fig. 1B). Hence, it seems that the formation of FFT during coffee roasting via 2-furaldehyde as intermediate compound is a minor pathway only. In addition, incorporation of D-[U-13C5]-arabinose did not yield fully labelled FFT as it would be expected, but partially labelled FFT with 13C1, 13C2 and 13C3-moieties (Figure 2). In line with these data, spiking of green coffee with arabinose did not result in increased amounts of FFT nor 2-furaldehyde, which confirms the conclusion of Milo et al. (6) that FFT is mainly formed from the non-water soluble fraction. In contrast, spiking experiment (Figure 1B) with cysteine resulted in enhanced FFT amounts, which shows an interesting avenue for coffee aroma modulation.

Figure 1. Omission experiments (A) as well as spiking of green coffee with sugars (equimolar amounts) and cysteine (B). Water exhausted beans (EB), biomimetic recombined extract (BRE).
Pyrazines. Omission and spiking experiments confirmed the importance of free amino acids as precursors for the alkylpyrazines (Figure 3). Omission of all free amino acids decreased the amounts of 2-ethyl-3,5-dimethylpyrazine (EDMP) and 2,3-diethyl-5-methylpyrazine (DEMP) to 50% and even less than 25%, respectively, relative to the reference (BRE). As a confirmation of this result, spiking with alanine highly increased amounts of pyrazines, EDMP to almost 300% and DEMP up to more than 900%. All these results are further sustained by the spiking experiment with labelled alanine, which was efficiently incorporated into the side chain of the DEMP molecule (Figure 2B).

To study the impact of free sugars on pyrazine formation, recombined green coffee was omitted in all water-soluble sugars. This led to a considerable increase in contents of the two pyrazines (Figure 3A). Together with the fact that spiking of green beans with sucrose and arabinose had a suppressing effect on alkylpyrazine contents (Figure 3B), it can be stated that their generation involves the competition between bound and free sugars for the water extractable nitrogen source.
arabinose having the highest potential in generating furan, followed by rhamnose and sucrose (Figure 4A).

Figure 4. Spiking of green coffee with different sugars (equimolar amounts) and/or amino acids (A) and incorporation of D-[U-13C₅]-arabinose (16% of natural content) (B).

References