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Low-field ^{1}H NMR spectroscopy for distinguishing between arabica and robusta ground roast coffees

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ABSTRACT

This work reports a new screening protocol for addressing issues of coffee authenticity using low-field (60 MHz) bench-top ^{1}H NMR spectroscopy. Using a simple chloroform-based extraction, useful spectra were obtained from the lipophilic fraction of ground roast coffees. It was found that 16-O-methylcafestol (16-OMC, a recognized marker compound for robusta beans) gives rise to an isolated peak in the 60 MHz spectrum, which can be used as an indicator of the presence of robusta beans in the sample. A total of 81 extracts from authenticated coffees and mixtures were analysed, from which the detection limit of robusta in arabica was estimated to be between 10% and 20% w/w. Using the established protocol, a surveillance exercise was conducted of 27 retail samples of ground roast coffees which were labelled as "100% arabica". None were found to contain undeclared robusta content above the estimated detection limit.

1. Introduction

Coffee beans are one of the most widely traded commodities in the world, and as such, are vulnerable to fraud within the supply chain (Toci, Farah, Pezza, & Pezza, 2016). The two main species grown are *Coffea arabica* L. (around 70% of the market) and *Coffea canephora Pierre ex A. Froehner* (variety robusta) (Belitz, Grosch, & Schieberle, 2009). Arabica beans are the most expensive, and are prized for their smooth, rounded flavour, whilst the more disease-resistant robusta plants produce beans that yield a rougher brewed drink, and thus command a lower price. There is potential, therefore, for unscrupulous traders to make economic gain by partially or wholly substituting arabica with robusta beans, deceiving other parties in the supply chain and, ultimately, the consumer. Objective methods are needed for the reliable identification of both species, and for the estimation of their contents in coffee products. Whole beans may be distinguished by inspection (International Coffee Organization, 2016; Mendonca, Franca, & Oliveira, 2009), but chemical analysis is required to confirm the identity of ground roast products, for example to detect the adulteration of arabica by amounts of robusta.

Coffee contains a complex mixture of hundreds of different organic compounds, present in concentrations ranging from trace quantities up to tens of percent by weight. Major components are carbohydrates, amino acids and lipids. Potentially more characteristic of the individual species, however, are minor components such as the diterpenes of the kaurane family, whose presence in different coffee products is relatively well-documented (Kurzrock & Speer, 2001; Scharnhop & Winterhalter, 2009). These include cafestol, found in both bean types, and kahweol, found in arabica beans and in some, but not all, robusta beans. A further diterpene, 16-O-methylcafestol (16-OMC), is found exclusively in robusta beans, and has thus been proposed as a reliable marker for distinguishing between the two bean types (Speer & Mischnick, 1989). The stability of 16-OMC with respect to the roasting process means that it can also be used to detect the presence of robusta in processed coffee products (Speer & Koelling-Speer, 2006). An official method exists for the determination of 16-OMC in roasted coffee by HPLC, but it requires a time-consuming sample preparation ("DIN 10779, 2011"). Alternative methods that are rapid and low-cost would increase the uptake of authenticity testing and be of benefit to the sector.

High-field ^{1}H NMR spectroscopy has been previously reported for the analysis of coffee. The majority of studies have examined aqueous extracts of coffee, in a variety of applications including

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ascertaining species and geographical origin (Cagliani, Pellegrino, Giugno, & Consonni, 2013; Charlton, Farrington, & Brereton, 2002; Consonni, Cagliani, & Cogliati, 2012; Schievano, Finotello, De Angelis, Mammi, & Navarini, 2014; Wei, Furihata, Hu, Miyakawa, & Tanokura, 2011; Wei et al., 2012). In contrast, a recent study (Monakhova et al., 2015) focused on the analysis of lipophilic extracts from coffee beans and products, and their potential for addressing issues of authenticity in arabica and robusta coffees. It was shown that many minor components, including kahweol and 16-OMC, produce clearly identifiable peaks in 400 MHz spectra, and further, that integration of the 16-OMC peaks can be used to estimate the amount of robusta in coffee blends with an approximate detection limit of 1–3% w/w. The authors concluded that high-field NMR spectroscopy has potential as a screening tool for identifying coffee species, for example in advance of applying the more time-consuming official method.

The present paper explores whether a recent development in NMR technology, low-field ("benchtop") spectroscopy, can similarly be used to address issues of coffee authentication. Compared with high-field instruments (Blümich (2016), Blümich, Casanova, and Appelt (2009)), benchtop spectrometers are smaller and more robust. Capital and maintenance costs are lower, as these instruments utilise permanent rather than superconducting magnets and thus do not need any cryogens. Modern benchtop spectrometers are also high-resolution instruments, capable of capturing as many data points per frequency interval as their high-field counterparts. However, their lower magnetic field strengths (typically 40–100 MHz) mean that resonances appear broader and more overlapped (Gerdova et al., 2015; Jakes et al., 2015). Although the chemical shifts of protons on the ppm scale are invariant to field strength, frequency separations (in Hz) are not. For instance, a chemical shift difference of 0.1 ppm translates into a separation of 60 Hz in a 600 MHz spectrum, but of only 6 Hz at an operating frequency of 60 MHz. Furthermore, second order effects on multiplet intensities are more important at lower fields, since chemical shift differences (in Hz) are reduced relative to J-couplings (typical J = 4–12 Hz: the J-coupling is invariant to field strength). Thus, when displayed on a conventional chemical shift scale, spectra that contain many resonances exhibit substantially different profiles at low- and high-field strengths. Consequently, it is not obvious that an analysis developed using high-field spectra will translate readily to low-field measurements.

As in the work by Monakhova et al. (2015), the present paper focuses on analysis of the lipophilic fraction extracted from samples of ground roast coffee beans. The aim has been to determine whether low-field NMR spectroscopy can offer the specificity and sensitivity needed to distinguish between arabica and robusta samples, and further, to quantitatively characterize mixtures of the two. Spectra obtained at both low (60 MHz) and high (600 MHz) field strengths are compared and contrasted, and the previously unreported low-field spectrum annotated. A protocol is described for detecting the presence of ground robusta beans in a sample, through a distinct spectral signature arising from the marker compound 16-OMC. Finally, results are reported from application of the low-field method to a collection of retail samples of ground roast coffees, all of which carried the labelling claim "100% arabica".

2. Materials and methods

2.1. Samples

17 samples of roast coffee beans were obtained from a range of UK retailers and from the British Coffee Association, as detailed in Table 1(a). The authenticity of these intact bean samples was confirmed by inspection. Combinations of these samples were used to produce an assortment of 54 mixtures, as detailed in Table 1(b). In addition, 27 samples of ground roast coffees, all of which displayed the labelling claim "100% arabica" on their packaging (and two of which were also labelled decaffeinated), were purchased from UK retailers (Table 1(c)). 16-OMC and deuterated chloroform were purchased from Sigma Aldrich (Gillingham, UK).

Table 1
Description of coffee samples.

(a) Whole bean samples

	Number of samples	Number of extracts	Comments
Arabica	7	14	Purchased from UK retailers. Includes one decaffeinated sample. Two extracts prepared per sample Geographic origins of the beans, as stated on labels: Kenya, Peru (×2), Indonesia, Africa & South America (blend), Africa & Central & South America (blend), 1 × origin not stated
	4	4	Supplied by the British Coffee Association Geographic origins of the beans: Colombia, Honduras, Nicaragua, Brazil
Robusta	3	6	Purchased from UK retailers. Two extracts prepared per sample Geographic origins of the beans, as stated on labels: India, Tanzania, 1 × origin not stated
	3	3	Supplied by the British Coffee Association Geographic origins of the beans: Vietnam, India, Uganda

(b) Mixtures prepared from whole bean samples

% w/w arabica	% w/w robusta	Number of samples	Number of extracts	Comments
90, 80, 70,... 10	10, 20, 30,... 90	18	18	Two mixture series, each prepared from a randomly selected pair of whole bean samples
90, 80, 60	10, 20, 40	36	36	Twelve partial mixture series, each prepared from a different pairwise combination of whole bean samples

(c) Surveillance samples (retail-purchased ground roast coffees)

	Number of samples	Number of extracts	Comments
Labelled "100% arabica"	27	32	Purchased from UK retailers. Includes two decaffeinated samples. Two extracts prepared from five of the samples Geographic origins of the beans, as stated on the labels: Indonesia, Central & South America (blend), Africa & Asia & South America (blend), Africa & Brazil & Central America (blend), Guatamala (×3), Latin America, Brazil, Indonesia & Africa & Latin America (blends, ×2), Sumatra, Java, Columbia (×2), Kenya, Java & Sumatra (×2), Costa Rica, "multiple countries of origin" (×3), origin not stated (×5)