Solid-state NMR spectroscopy of roasted and ground coffee samples: evidences for phase heterogeneity and prospects of applications in food screening

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Abstract – The advancement in the use of spectroscopic techniques to investigate coffee samples is of high interest especially considering the widespread problems with coffee adulteration and counterfeiting. In this work, the use of solid-state nuclear magnetic resonance (NMR) is investigated as a means to probe the various chemically-distinct phases existent in roasted coffee samples and to detect the occurrence of counterfeiting or adulterations in coffee blends. Routine solid-state ^{1}H and ^{13}C NMR spectra allowed the distinction between different coffee types (Arabica / Robusta) and the evaluation of the presence of these components in coffee blends. On the other hand, the use of more specialized solid-state NMR experiments revealed the existence of phases with different molecular mobilities (e.g., associated with lipids or carbohydrates). The results illustrate the usefulness of solid-state NMR spectroscopy to examine molecular mobilities and interactions and to aid in the quality control of coffee-related products.

Keywords – Solid-state NMR; Roasted and ground coffee; Food composition; Adulteration.

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1. Introduction

Coffee is one of the most sought-after commodities in the world (International Coffee Organization, 2021). As such, there is a huge interest in the development of efficient methods that can be successfully employed for screening and quality control of coffee beans and coffee-derived products. The deep understanding about the chemical composition of coffee is relevant not only from the food science point of view, but also due to the pharmacological relevance of many of its components (e.g., caffeine, chlorogenic acids and diterpenes) (Esquivel & Jiménez, 2012; Cano-Marquina, Tarín, & Cano, 2013). There are two commercially relevant coffee species, *Coffee arabica* (known as Arabica coffee) and *C. canephora* (known as Robusta or Conilon coffee) (Finotello, Forzato, Gasparini, Mammi, Navarini, & Schievano, 2017). Beverages obtained from Arabica coffee generally exhibit richer taste and aroma as compared to Robusta, which means that Arabica coffee has a higher value in the market than the Robusta form. Therefore, adulteration of the more expensive form with the cheaper one can lead to fraudulent commercial gain. In contrast with intact coffee beans, it is more difficult to find out the authenticity of ground roasted coffee upon inspection. Therefore, it is highly desirable to establish efficient tools that can allow the detection and quantification of the presence of Robusta coffee in commercial products labelled as "100 % Arabica". Many efforts have been taken in this way, involving the use of spectroscopic methods – e.g., mass spectrometry, liquid chromatography, ultraviolet-visible (UV-Vis), Fourier-transform infrared (FTIR), near infrared (NIR) and solution ^{1}H nuclear magnetic resonance (NMR) spectroscopies – to discriminate Arabica and Robusta varieties in both raw and ground roasted coffee samples (Kemsley, Ruault, & Wilson, 1995; Monakhova et al., 2015; Defernez et al., 2017; Hong et al., 2017; Correia et al., 2018; Gunning et al., 2018). Furthermore, methods as these have also been applied aiming the detection of several types of adulterants fraudulently mixed with roasted coffee (again to obtain cheaper mixtures), such as corn, barley, rice, soybean, wheat, chickpea and coffee husks (Toci, Farah, Pezza, & Pezza, 2016; Hong et al., 2017; Ribeiro, Boralle, Pezza, Pezza, & Toci, 2017; Sezer, Apaydin, Bilge, & Boyaci, 2018; Milani, Rossini, Catelani, Pezza, Toci, & Pezza, 2020).

Solution ^{1}H and ^{13}C NMR spectroscopy has been used as a screening protocol in many studies of coffee products, including raw and roasted coffee beans (Bosco, Toffanin, De Palo, Zatti, & Segre, 1999; Wei et al., 2012; Monakhova et al., 2015; Defernez et al., 2017; Finotello et al., 2017; Ribeiro et al., 2017; Hong et al., 2017; Gunning et al., 2018; Milani et al., 2020). The main benefits of NMR spectroscopy in comparison with other spectroscopic methods include the ease of sample preparation (with no need of time-consuming purification or chemical derivation stages), the quantitative character of the technique and the high resolution of the NMR spectra that allow the identification of the components present in the solution (Bosco et al., 1999; Wei et al., 2012). Solid-state NMR spectra have inferior resolution in comparison with spectra recorded in solution, so the applicability of solid-state NMR spectroscopy to studies involving coffee samples is somewhat limited. Nevertheless, some examples can be found in the literature where solid-state NMR spectra have been exploited to obtain information on chemical aspects of coffee beans and other coffee-related materials (Nogueira, Boffo, Tavares, Moreira, Tavares, & Ferreira, 2011; Low, Rahman, & Jamaluddin, 2015; Kanai, Yoshihara, & Kawamura, 2019). For instance, a recent report has described the use of solid-state ^{1}H and ^{13}C NMR spectra to assess the contributions due to lipids and polysaccharides in raw coffee beans, roasted coffee beans and spent coffee grounds, noting that these lipids are a potential source for the production of biofuels (Kanai et al., 2019). The most outstanding advantage of solid-state NMR in comparison with solution NMR experiments refers to the possibility of conducting the experiments with essentially no sample preparation or modification; the material to be analysed (e.g., raw coffee beans, roasted coffee beans or granules and spent coffee grounds) can be directly powdered and packed into the NMR rotor, so as to obtain spectra representative of the intact sample. In this regard, the development of solid-state NMR spectroscopy methods that can be used in studies of intact coffee products is of high interest.

In this work, the use of solid-state NMR spectroscopy is exploited in detail to study roasted coffee samples, with special emphasis on the assessment of the chemically heterogeneous nature of products of the Arabica and Robusta variants. The main hypothesis to be examined here is that solid-state

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77 NMR spectroscopy can be used effectively to assess the chemical composition of roasted coffee

78 samples, without any chemical modifications, and are also useful for screening purposes to detect

79 counterfeiting of coffee products. Whereas many previous NMR studies dealing with coffee products

80 have been conducted in solution (with the extraction of hydrophilic or lipophilic components of

81 coffee), the present investigation involves solid-state NMR experiments conducted with intact roasted

82 and ground coffee samples. With the use of pulse sequences suitable to emphasize the different

83 responses of phases with distinct dynamics present in the material, the contributions due to lipids and

84 carbohydrates are identified in ^{1}H and ^{13}C NMR experiments carried out with intact samples. As

85 detailed later, the identification of the signals due to lipids is particularly important for authentication

86 purposes, since Arabica and Robusta varieties exhibit different amounts of lipids and also distinct

87 types of diterpenes present in the lipid fraction. The solid-state NMR experiments are non-destructive,

88 relatively fast and straightforward to implement in commercial NMR spectrometers equipped with

89 conventional solid-state probes. Moreover, the potential of solid-state NMR spectroscopy to allow

90 the distinction of the contributions from Arabica and Robusta coffee variants in coffee blends is also

91 demonstrated, illustrating the usefulness of this approach for screening and identification of

92 counterfeit coffee products.

93

94 **2. Experimental methods**

95 2.1 *Samples*

96 Samples of Arabica and Robusta ground roasted coffee beans were obtained from the state Minas

97 Gerais (MG), in southeast Brazil. From these samples, seven mixtures were prepared with differing

98 amounts of Arabica and Robusta coffee variants (i.e., with 0, 5, 25, 50, 75, 95, and 100 wt. % of

99 Arabica coffee in the mixture). The coffee samples were gently ground using a mortar and pestle into

100 a fine and homogenous powder, which was packed into a 4 mm NMR rotor; the mass of sample inside

101 the rotor was in the range 50-60 mg.

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103   2.2 *NMR experiments*

104   Solid-state NMR experiments were conducted at room temperature in a 300 MHz Bruker Avance

105   spectrometer III running the Topspin 3.2 software. The powdered coffee samples were packed into

106   4 mm diameter zirconia rotors for magic angle spinning (MAS) experiments at the frequency of

107   12 kHz. Both ^{1}H and ^{13}C NMR experiments were conducted, at frequencies of 300.13 and

108   75.47 MHz, respectively. The one-dimensional (1D) spectra were obtained by Fourier transform of

109   the free induction decays (FIDs), after zero filling (twice) and exponential line broadening of 1 and

110   50 Hz for ^{1}H and ^{13}C NMR spectra, respectively. The chemical shifts were expressed in parts per

111   million (ppm) and were referenced to tetramethylsilane (TMS), using adamantane and

112   hexamethylbenzene (HMB) as secondary references for ^{1}H and ^{13}C NMR spectra, respectively.

113   Different sets of experiments were conducted for each probe nucleus, as described in the sequence.

114   The ^{1}H NMR spectra were recorded using single pulse (SP) excitation, with a $\pi/2$ pulse duration of

115   3.5 μs, a recycle delay of 5 s, a spectral window of 12 kHz (or 100 kHz in some cases, to allow the

116   recording of the spinning sidebands) and the accumulation of 256 scans. In order to suppress the

117   contribution from rigid components with short transverse relaxation time (T_2), which are responsible

118   for the production of strong and broad resonances affecting the baseline underneath the narrow signals

119   due to the more mobile components, a "T_2 filter" was included before the $\pi/2$ excitation pulse,

120   composed by the block of pulses: $(\pi/2)_x - \tau - (\pi)_y - \tau$ (Mackenzie & Smith, 2002; Rastrelli, Jha, &

121   Mancin, 2009). The time delay τ was set at 1 ms, after several tests conducted to reach a compromise

122   between the suppression of the broad contributions and the overall signal loss due to transverse

123   relaxation. The longitudinal relaxation time (T_1) was measured selectively for the mobile components

124   by inserting the following block of pulses after the T_2 filter and just before the $\pi/2$ excitation pulse:

125    $(\pi/2)_{\pm x} - t_{REC} - (\pi/2)_{\mp x}$. This type of block pulse is commonly used to study spin diffusion in polymers

126   and other materials (Kumashiro, Schmidt-Rohr, Murphy, Ouellette, Cramer, & Thompson, 1998); the

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127 first $\pi/2$ pulse in the block transfers the nuclear magnetization (which survived after the T_2 filter) to

128 the z axis; after the recovery time (t_{REC}), the magnetization is transferred back to the transverse plane

129 by the second $\pi/2$ pulse (with inverted phase), for detection. In the absence of spin diffusion (which

130 was not significant in the experiments involving the coffee samples studied here, as described later),

131 the detected signal is attenuated only by longitudinal relaxation occurring during the interval t_{REC}.

132 Thus, this method allows the selective study of the longitudinal relaxation process of the mobile

133 components present in the material.

134 As for the ^{13}C nuclei, the ^{1}H-^{13}C cross polarization (CP) experiments comprised the use of a $\pi/2$ ^{1}H

135 excitation pulse of 3.5 μs, a contact time of 1000 μs (chosen after optimization of the overall signal

136 intensity), a recycle delay of 5 s, a spectral window of 50 kHz and the accumulation of 1024 scans.

137 A linear ("ramped") variation of the RF amplitude was included in the ^{1}H channel during the contact

138 time and high-power ^{1}H decoupling with small phase incremental alteration in 64 steps (SPINAL-64)

139 was employed during the FID detection. In order to investigate the contributions of species with

140 distinct molecular mobilities to the ^{13}C NMR spectra, experiments combining cross polarization and

141 single pulse excitation (known as CP-SP experiments) were also conducted (Shu, Li, Chen, & Zhang,

142 2010). In these experiments, a $\pi/2$ ^{13}C excitation pulse of 4.5 μs was added just before the contact

143 time. The spectra recorded in the CP-SP experiments are expected to contain contributions from both

144 the rigid and the mobile components, whereas the CP spectra typically contain just the contributions

145 from the rigid components, since molecular motion averages out the dipolar coupling between the ^{1}H

146 and ^{13}C nuclei (Shu et al., 2010; Courtier-Murias et al., 2014). This selectivity of the CP process

147 towards the rigid components also allowed the indirect measurement of the T_1 values for the ^{1}H nuclei

148 in these moieties; for this, a saturation-recovery experiment was conducted by inserting a saturation

149 train containing 4 $\pi/2$ pulses separated by 1 ms in the ^{1}H channel, followed by variable recovery

150 intervals, prior to the polarization transfer to the ^{13}C nuclei.

151 Two-dimensional (2D) ^{1}H-^{13}C CP-based wideline separation (WISE) experiments were also

152 performed, in order to investigate the correlation between ^{1}H NMR signals due to groups with

153 different molecular mobilities and chemically distinct ^{13}C resonances (Schmidt-Rohr et al., 1992).

154 The same values of contact time, ^{1}H excitation pulse length and recycle delays used in the 1D CP

155 experiments were also used in the 2D WISE experiments. The spectral window in the indirect

156 dimension (F$_1$) was 100 kHz, with a total of 128 increments of the evolution time (t_1) and

157 accumulation of 128 scans for each t_1 value.

158

3. Results and discussion

160 Typical ^{1}H MAS NMR spectra obtained for a ground coffee sample (containing 100 wt. % Arabica

161 coffee) are shown in Fig. 1. These spectra were recorded with a large spectral window (100 kHz), so

162 that first-order spinning sidebands are clearly identified. The spectrum recorded with a conventional

163 single pulse excitation experiment (shown in red) contains contributions from both the rigid and the

164 mobile components; the rigid components (mostly corresponding to carbohydrates) give rise to

165 extremely broad resonances, including the centerband and the spinning sidebands. For these

166 components, the MAS rate used in the NMR experiments (12 kHz) is not sufficiently high to suppress

167 the strong ^{1}H-^{1}H dipolar couplings, leading to the observation of a considerable homogeneous

168 broadening that limits the overall spectral resolution. Such broad contributions appear in the ^{1}H NMR

169 spectrum as a featureless background, on the top of which the narrow signals due to the mobile

170 components (associated with lipids) appear. It is worth noting that spinning sidebands are also

171 observed for these narrow signals, indicating that the removal of the anisotropic broadening by

172 molecular motion is just partial and so these components cannot be considered "liquid-like", as it has

173 been suggested in previous investigations dealing with raw and roasted coffee beans (Kanai et al.,

174 2019).

175 Also, in the work by Kanai et al. (2019), a broad component centered around 4.5 ppm in the ^{1}H NMR

176 spectra of raw and roasted coffee beans has been attributed to the occurrence of water molecules with

177 limited mobility. The broad signals observed in the present study in the spectra recorded with a

178 conventional single pulse excitation experiment (shown in red in Fig. 1) also have the centerband

179 occurring at similar chemical shifts; however, the origin of these broad signals is unambiguously

180 identified as related to carbohydrates, as it will become clear in the discussion of the 2D [1]H-^{13}C CP-

181 based WISE spectra (see below).
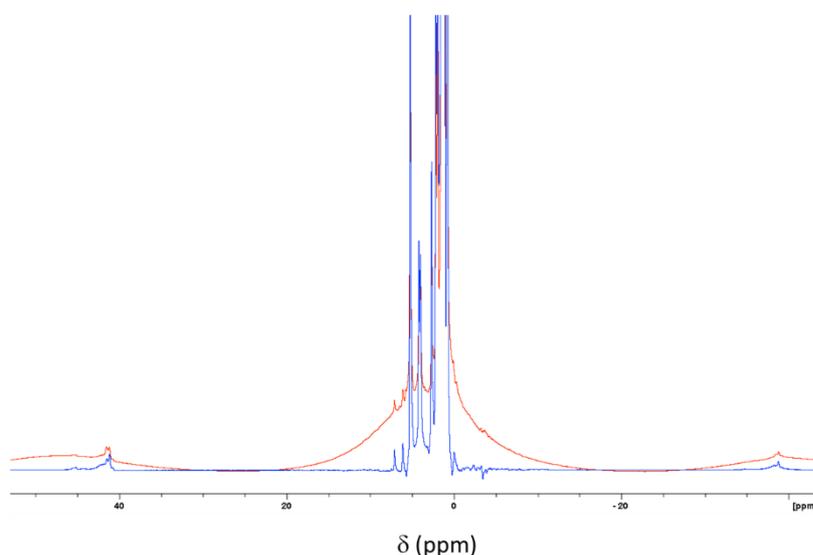
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183 **Figure 1 –** ^{1}H MAS NMR spectra recorded for the sample containing 100 wt. % Arabica coffee and

184 exhibited in a wide spectral range, including (blue line) or not (red line) the T_2 filter before the $\pi/2$

185 excitation pulse.

186

187 With the use of the T_2 filter, the contributions due to the mobile components in the ^{1}H NMR spectra

188 of the coffee samples are observed with a remarkably improved resolution, as illustrated in Fig. 2.

189 This high resolution is not usual for ^{1}H NMR spectra recorded at moderate MAS rates in solid samples

190 with no homonuclear decoupling scheme (Lesage, 2009), but similar spectra have been previously

191 reported for raw and roasted coffee beans (Nogueira et al., 2011; Kanai et al., 2019). As mentioned

192 above, the observation of these narrow lines is a direct indication of the highly mobile character of

8

193 the lipid molecules present in the coffee samples. It is worth noting that Kanai et al. (2019) reported

194 the detection of ^{1}H NMR spectra containing only the narrow contributions for the liquid lipid residue

195 obtained after extraction with *n*-hexane, which was obviously free of carbohydrate contributions. In

196 the present work, the use of the T_2 filter led to the achievement of ^{1}H NMR spectra containing just

197 the lipid contributions in a straightforward way, which thus allows the possibility of obtaining detailed

198 chemical information about these moieties without the need of laborious extraction methods.

199 The ^{1}H NMR spectra shown in Fig. 2 are representative of the triacylglycerides (TAGs) present in

200 coffee. The detected signals are similar to the ones present in ^{1}H NMR spectra recorded in solution

201 for several types of edible oils, due to the structural resemblance of the fatty acids present in coffee

202 and in other sources such as corn, olive and hazelnut oils (D'Amelio, De Angelis, Navarini,

203 Schievano, & Mammi, 2013; Parker, Limer, Watson, Defernez, Williamson, & Kemsley, 2014). A

204 detailed assignment of the chemical shifts identified in the ^{1}H NMR spectra of coffee samples has

205 been provided before, indicating that the TAGs present in coffee involve mixtures mostly containing

206 saturated fatty acids, oleic and linoleic acids (D'Amelio et al, 2013; Kanai et al., 2019). The dominant

207 peak in these spectra occurs at 1.3 ppm, due to chain methylene groups, whereas other easily

208 identifiable signals include terminal methyl, allylic, bis-allylic, glyceride and olefinic groups (see Fig.

209 2a).
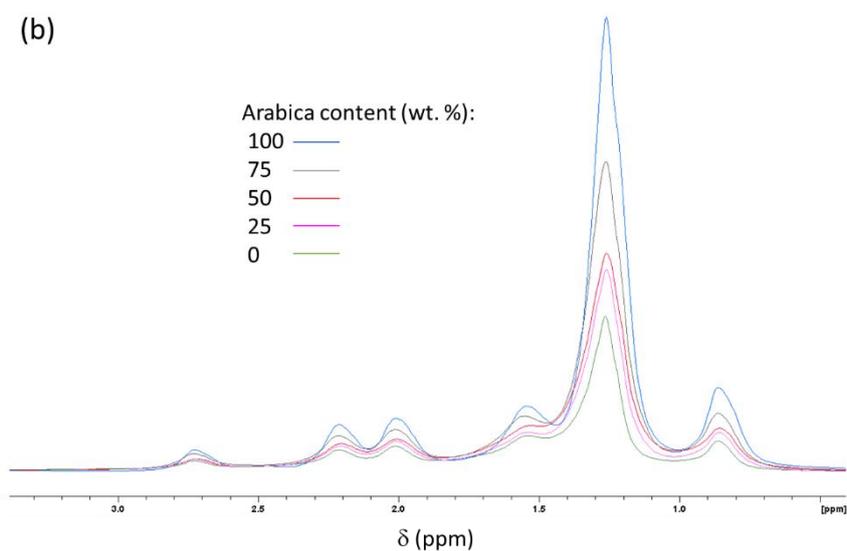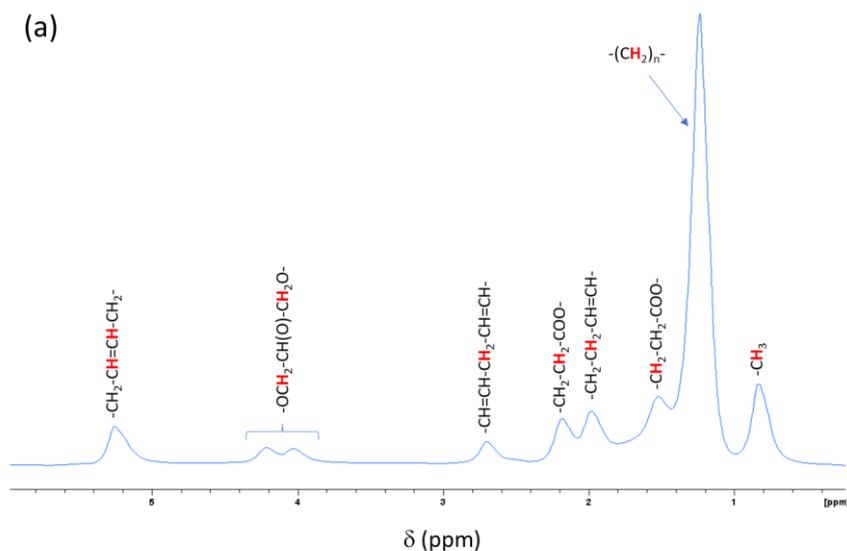
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212 **Figure 2** – ^{1}H MAS NMR spectrum recorded with the T_2 filter before the $\pi/2$ excitation pulse for the

213 sample containing 100 wt. % Arabica coffee, with indication of the chemical groups responsible for

214 the dominant signals (a), and for a set of samples containing mixtures of Arabica and Robusta coffee

215 in different proportions (b).

216

217 All these resonances are common for the TAGs present in both types of coffee analysed in this work;

218 the main differences between the Arabica and Robusta varieties in this regard correspond to the

219 amount of lipids, which is considerably higher in Arabica coffee (Speer & Kölling-Speer, 2006;

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220 D'Amelio et al, 2013). Consequently, the absolute intensity of the narrow signals in the ^{1}H NMR

221 spectra obtained for the coffee mixtures (after correcting for the different sample masses used in each

222 experiment) shows a steady increase as a function of the Arabica content in the mixture, as illustrated

223 for a representative set of samples in Fig. 2b. It is worth noting that these spectra were recorded using

224 exactly the same experimental conditions and the intensities were normalized by the sample masses,

225 so that the absolute intensities can be reliably compared from sample to sample. The quantitative

226 character of this comparative analysis could be improved by using an internal reference standard (with

227 a reference peak with known intensity) or an electronically-synthesized reference signal, as

228 commonly done in quantitative NMR (Bharti & Roy, 2012). But, even without these procedures, the

229 results shown in Fig. 2b clearly demonstrate how the distinct lipid contents of Arabica and Robusta

230 coffee lead to significant differences in the strong peaks present in the solid-state ^{1}H NMR spectra

231 recorded with the T_2 filter.

232 On the other hand, there are some less intense signals obtained in solid-state ^{1}H NMR experiments

233 that can be used to distinguish the Arabica and Robusta varieties, due to the differences in the

234 chemical composition of the lipids. In fact, the signals associated with coffee diterpenes such as

235 kahweol, cafestol and 16-O-methylcafestol (16-OMC) have been used in previous solution NMR

236 investigations as indicators of the presence of Arabica or Robusta varieties and thus are useful for

237 studies aiming authenticity checks of coffee mixtures (Monakhova et al., 2015; Defernez et al., 2017;

238 Finotello et al., 2017; Gunning et al., 2018). Among these, kahweol is known to be present mostly in

239 Arabica forms and is less common in Robusta, whereas cafestol is common in both varieties. On the

240 other hand, 16-OMC has long been thought to occur only in Robusta coffee and thus considered as a

241 useful marker for coffee authenticity, also because this compound is stable during the roasting process

242 (Speer & Kölling-Speer, 2006; D'Amelio et al, 2013; Defernez et al., 2017). However, a recent work

243 has disputed this consensus by claiming that 16-OMC is also present in Arabica, albeit in very small

244 amounts (Gunning et al., 2018). All these dipertenes produce signals that can be easily identified in

245 high-resolution ^{1}H NMR spectra of lipophilic coffee extracts (D'Amelio et al, 2013; Monakhova et

246 al., 2015), even at low magnetic fields (Defernez et al., 2017). Specific signals due to kahweol and

247 cafestol appear in the range 5.9-7.3 ppm, whereas 16-OMC gives rise to a well-defined signal around

248 3.16 ppm in solution ^{1}H NMR spectra obtained for coffee oil and lipophilic coffee extracts (D'Amelio

249 et al., 2013; Defernez et al., 2017).

250 The possibility of detection of the signals due to kahweol, cafestol and 16-OMC in solid-state ^{1}H

251 NMR spectra of roasted coffee samples is demonstrated in Fig. 3, taking advantage of the significant

252 improvement in resolution obtained due to the use of the T_2 filter. Clear differences are observed

253 between the signals observed for pure Arabica and pure Robusta; moreover, a progressive change in

254 these signals is observed for the mixtures containing different amounts of each coffee variety. The

255 peak around 3.15 ppm (due to methyl groups in 16-OMC) shows a steady growth with the increase

256 in the amount of Robusta coffee in the mixtures. On the other hand, the resonances between 6.0 and

257 7.5 ppm are significantly more intense for the samples containing more amount of Arabica coffee,

258 which is consistent with the higher oil content of these samples (see also Fig. 2b); the number of

259 signals detected in this range and their chemical shifts also show a continuous change with the

260 increase in the Arabica coffee amount in the mixtures, which is a direct consequence of the differences

261 in diterpene composition of Robusta and Arabica coffee varieties (Speer & Kölling-Speer, 2006;

262 D'Amelio et al, 2013; Defernez et al., 2017). Thus, since the solid-state ^{1}H NMR spectra of coffee

263 samples are completely dominated by the contribution due to coffee oil and considering that the main

264 signals useful to differentiate Arabica and Robusta varieties are due to the diterpenes present in the

265 lipid fraction, this method can indeed be useful for screening studies aiming to assess the authenticity

266 of coffee products. This could be facilitated using chemometric multivariate methods, similarly to

267 what has been done using solution-state NMR experiments with lipophilic coffee extracts (Defernez

268 et al., 2017; Gunning et al., 2018).

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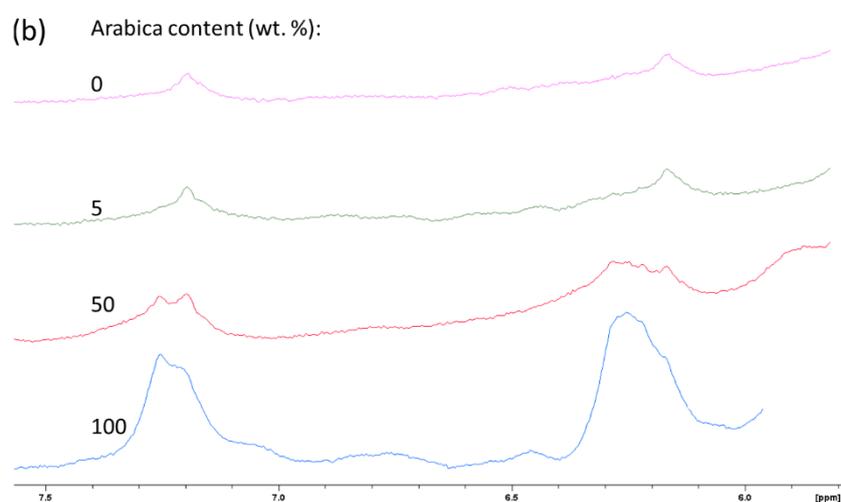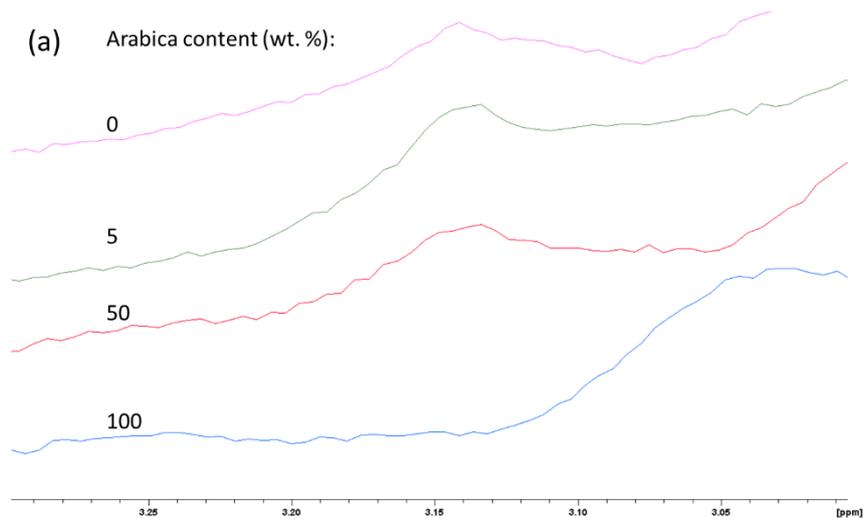
Figure 3 – ^{1}H MAS NMR spectra recorded with the T_2 filter before the $\pi/2$ excitation pulse for a set of samples containing mixtures of Arabica and Robusta coffee in different proportions, highlighting the chemical shift ranges containing typical signals due to diterpenes 16-OMC (a), cafestol and kahweol (b).

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It is worth stressing at this point some important advantages of solid-state ^{1}H NMR spectroscopy regarding the possibility of identification of the signals due to 16-OMC for coffee authenticity applications. First of all, there is the obvious practical benefit of analyzing the coffee samples as received, with no need to dilute or obtain lipophilic extracts (as required in solution NMR experiments). Furthermore, several previous works have documented that the 16-OMC compound is not stable in chloroform solutions, especially when exposed to light, and thus the solution ^{1}H NMR spectra need to be recorded ideally within hours after extraction (D'Amelio et al., 2013; Defernez et al., 2017). In the case of ^{1}H solid-state NMR experiments reported here, no extraction is required and the presence of the 16-OMC marker can be analysed without destroying the structure of the coffee beans / powders, with no need to protect the sample to avoid degradation. The ^{1}H NMR spectra shown in Fig. 3 are fully reproducible and do not change over time, which is a huge advantage from the practical point of view.

The coexistence of phases with markedly different mobilities can also be assessed by solid-state ^{13}C NMR spectroscopy. As mentioned before, the ^{13}C NMR spectra of coffee samples obtained with CP are expected to be dominated by signals due to rigid components in carbohydrates, whereas the mobile components (in lipids) should contribute preferentially to the CP-SP spectra (Shu et al., 2010; Courtier-Murias et al., 2014). These features are clearly observed in the comparison shown in Figure 4, where the ^{13}C CP and CP-SP MAS NMR spectra are compared for the pure Arabica coffee sample; similar findings were also observed for the other coffee samples. In these spectra, the somewhat broad signals around 66, 74, 105 and 172 ppm are due to carbohydrates (mostly cellulose and hemicellulose), as it is commonly found in many different types of lignocellulosic materials (Cipriano, Chinelatto Jr., Nascimento, Rezende, de Menezes, & Freitas, 2020). In the case of coffee, these carbohydrates constitute the fibers present in the cell walls in the coffee beans (Kanai et al., 2019; Kanai et al., 2020).

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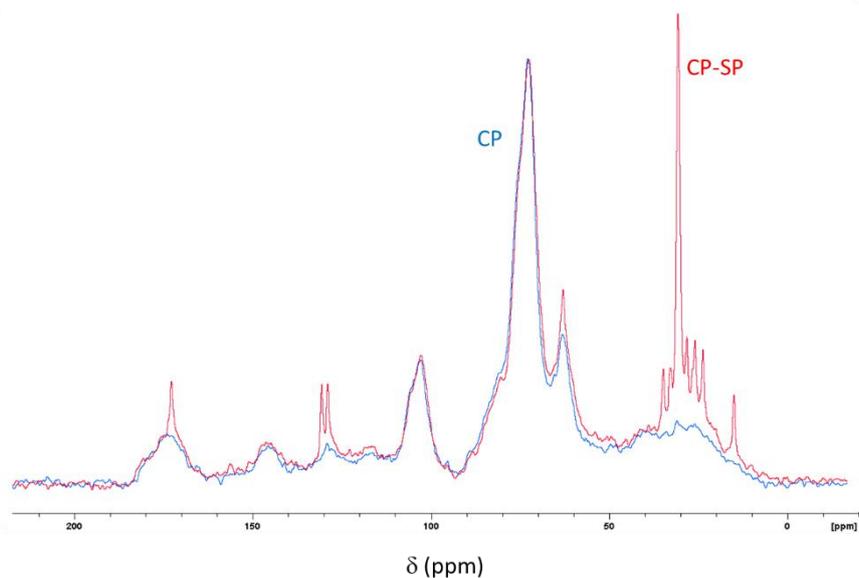
Figure 4 – Comparison of ^{13}C CP (blue line) and CP-SP (red line) MAS NMR spectra recorded for the sample containing 100 wt. % Arabica coffee.

Besides these resonances, the ^{13}C CP-SP MAS NMR spectra (Fig. 4) also contain a number of narrow peaks, similar to the ones observed in the ^{1}H MAS NMR spectra (Fig. 2). These lines are once more ascribed to the lipid moieties, which exhibit large molecular mobility and thus are unable to produce efficient ^{1}H-^{13}C polarization transfer, making impossible their full observation in the CP spectra. The chemical shifts of the narrow signals are consistent with previous results obtained for oils derived from coffee and other sources, showing the occurrence of TAGs composed of mixtures of saturated fatty acids, oleic and linoleic acids (D'Amelio et al., 2013; Kanai et al., 2019). The most intense narrow peaks in the CP-SP spectra are observed in the range 10-40 ppm, due to aliphatic CH_3 and CH_2 groups in the fatty acid chains (with the strongest CH_2 peak appearing at 32 ppm); other narrow peaks appear around 63 ppm (O-alkyl groups), 125 and 128 ppm (olefinic carbons), and 172 ppm (ester carbonyl groups).

316   The relative intensities of these narrow peaks in the CP-SP spectra show a steady growth with the

317   increase in the amount of Arabica coffee in the mixtures, as shown in Fig. 5, which is obviously a

318   consequence of the larger oil concentration in Arabica coffee in comparison with the Robusta variety.

319   This type of correlation points thus to another possibility (now using solid-state ^{13}C CP-SP MAS

320   NMR experiments) for the development of quantitative methods to determine the amount of Arabica

321   coffee in blends, which should be explored in future investigations.

322   The heterogeneous character of the coffee samples investigated in this work can be further assessed

323   using solid-state NMR experiments sensitive to the dynamic aspects related to each phase present in

324   the material. First, spin-lattice relaxation time measurements are a useful tool to probe the space

325   proximity between chemical groups belonging to phases with different mobilities. Due to the process

326   of spin diffusion mediated by the homonuclear dipolar coupling between ^{1}H nuclei, the corresponding

327   T_1 values associated with spatially close spins in rigid domains tend to become the same; for organic

328   materials, the domain size corresponding to a uniform T_1 value has been estimated in the range of

329   tens of nanometers (Aso et al., 2007). On the other hand, systems exhibiting phase separation (i.e.,

330   with domain sizes above this range) are expected to display distinct ^{1}H T_1 values corresponding to

331   each phase (when these values are distinct for the pure phases). Approaches based on relaxation time

332   and/or spin diffusion measurements have long been used to study phase separation and to estimate

333   domain sizes in polymer blends, pharmaceutical dispersions and biological systems, among others

334   (Clauss, Schmidt-Rohr, & Spiess, 1993; Duan et al., 2020).

(a)

δ (ppm)

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(b)

δ (ppm)

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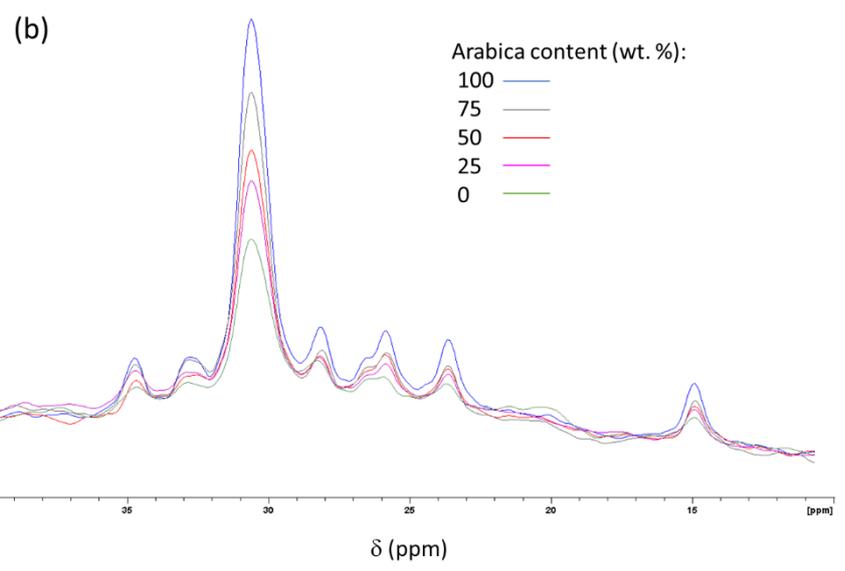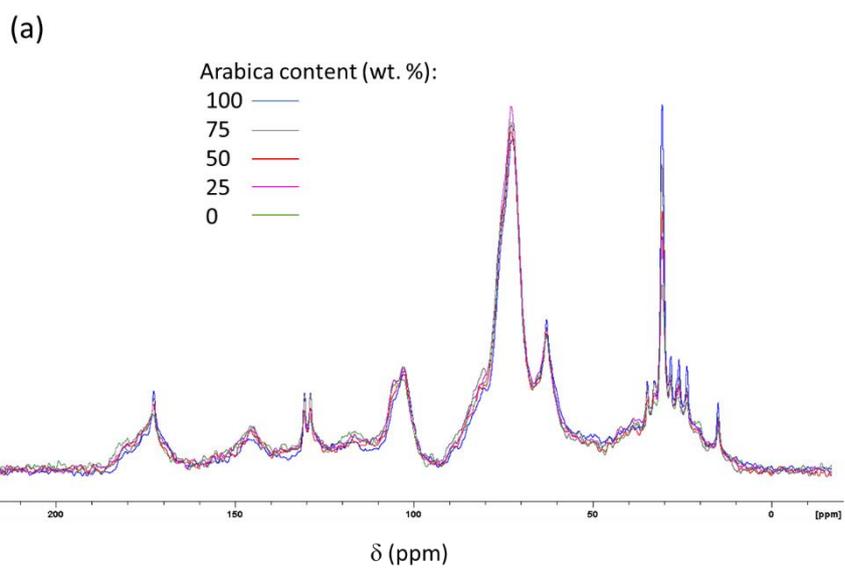
Figure 5 – ^{13}C CP-SP MAS NMR spectra recorded for a set of samples containing mixtures of Arabica and Robusta coffee in different proportions, shown in full range (a) and in the spectral range corresponding to the lipid signals (b).

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In the case of the coffee samples described here, the ^{1}H T_1 values were measured separately for the mobile and rigid components for a representative sample (containing 100 wt. % Arabica coffee), using the methods described in Section 2.2. The ^{1}H T_1 value directly measured for the mobile components (using T_2-filtered ^{1}H NMR spectra) was $T_1^{mobile} = 0.48$ s. On the other hand, the ^{1}H T_1

value of the rigid components (measured indirectly via ^{13}C-detected ^1H saturation-recovery experiments, using ^{13}C CP MAS NMR spectra) was $T_1^{rigid} = 0.88$ s. This non-uniformity in the spin-lattice relaxation rates thus indicates that there is no fine mixing of the rigid and mobile components detected in the solid-state ^1H and ^{13}C NMR spectra of the coffee samples. This finding is consistent with the occurrence of the mobile and rigid components (associated with lipids and carbohydrates, respectively) in well-separated domains, as expected from the well-known morphological characteristics of the coffee beans, where oil-rich regions and cell walls exhibit sizes typically in the micron range (Kasai, Konishi, Iwai, & Maeda, 2006; Kanai et al., 2019).

Similarly, experiments aimed at studying the possibility of ^1H polarization transfer from the mobile components to the rigid ones were tried, revealing unsuccessful results. This type of experiments has been shown to be very useful to investigate the polarization transfer mechanism in lipid bilayers (Kumashiro et al., 1998), plant cell walls (White, Wang, Park, Cosgrove, & Hong, 2014) and starch hydrogels (Koev, Muñoz-García, Iuga, Khimyak, & Warren, 2020), for instance. In the case of the T_2-filtered ^1H NMR spectra of the coffee samples, if there were an effective interaction between the ^1H nuclei in the oil-rich and carbohydrate-rich phases (e.g., involving spin diffusion or chemical exchange), the signal due to the rigid components (removed with the use of the T_2 filter) could be recovered after a mixing time; likewise, the insertion of a T_2 filter followed by a spin diffusion interval previous to the contact time in the ^{13}C CP MAS NMR experiments could reveal a recovery of the ^{13}C NMR signal due to the rigid components (White et al., 2014). The fail to observe such recovery either in the ^1H or the ^{13}C NMR experiments (even employing mixing times up to hundreds of ms) is a further corroboration of the scheme of two well-separated phases described above for the mobile and rigid components in the coffee samples.

This conclusion is further corroborated by the analysis of the 2D ^1H-^{13}C WISE results, illustrated in Fig. 6 for a representative coffee sample. In these experiments, the ^1H-^{13}C polarization transfer allows the correlation of ^{13}C NMR spectra directly observed in the F_2 dimension with the ^1H NMR wideline spectra indirectly detected along the F_1 dimension – with the latter being composed only of signals

due to ^{1}H nuclei that are effective in cross-polarizing the ^{13}C nuclei (Schmidt-Rohr et al., 1992). The

first noteworthy point in the plot shown in Fig. 6 is the dissimilarity between the ^{1}H (F_1) projection

of the 2D WISE spectra and the directly detected ^{1}H NMR 1D spectra shown in Figs. 1 and 2. Whereas

the 1D spectra are dominated by the narrow signals associated with lipids (mobile components), the

^{1}H (F_1) projection of the 2D WISE spectra comprises a quite broad and featureless resonance, nearly

matching the broad signal observed in Fig. 2, which was attributed to the ^{1}H nuclei in rigid

carbohydrate components. This finding is consistent with the ineffectiveness of the CP process for

the highly mobile lipid groups, so the contributions due to the ^{1}H nuclei in carbohydrates dominate

the F_1 projection of the WISE spectra. This result is similar to previous reports involving polymers

(Schmidt-Rohr et al., 1992), cellulose (Ali, Apperley, Eley, Emsley & Harris, 1996), soil samples

(Jäger, Schaumann & Bertmer, 2011) and porous carbon materials (Lopes et al., 2017).

Furthermore, the comparison between the plots in Fig. 6 and in Fig. 4 shows that, as expected, the

^{13}C (F_2) projections of the 2D WISE spectra are quite similar to the ^{13}C CP MAS NMR spectra, which

are dominated by contributions due to the rigid carbohydrate moieties. There are clear correlations

between the ^{13}C signals at 66, 74 and 105 ppm and the maximum of the ^{1}H (F_1) projection around

4.5 ppm – a chemical shift consistent with ^{1}H nuclei in carbohydrates (Ali et al., 1996). This finding

corroborates the interpretation given above about the origin of the broad signals observed in the ^{1}H

NMR spectra recorded with no T_2 filter (see Fig. 1). On the other hand, the narrow signals observed

around 30 ppm in the ^{13}C (F_2) projection of the 2D WISE spectrum of Fig. 6 exhibits a correlation

with a shoulder of the ^{1}H (F_1) projection at a lower chemical shift (*ca.* 1.0 ppm). This value is close

to the shifts of the strong and narrow signals observed in the 1D ^{1}H NMR spectra (see Figs. 1 and 2),

attributed to CH_2 groups in lipids; similarly, the chemical shift of 30 ppm corresponds to the narrow

peaks observed in the ^{13}C CP-SP MAS NMR spectra (Fig. 4). Thus, this correlation indicates the

existence of residual dipolar couplings that allow the observation of the lipid signals (although with

reduced intensity) in the 2D WISE spectra, evidencing that the high molecular mobility of these

moieties does not completely preclude the polarization transfer from the ^{1}H nuclei to the ^{13}C nuclei.

397　　It is clear, however, that this mobility causes a significant intensity reduction in the lipid signals, as

398　　already observed in the comparison between the CP and CP-SP spectra shown in Fig. 4. No

399　　correlation between the signals due to lipids and carbohydrates is detected in the 2D WISE spectra,

400　　reinforcing once more the scenario of two well-separated phases with marked distinct molecular

401　　mobilities for these chemical species in the coffee samples here investigated.
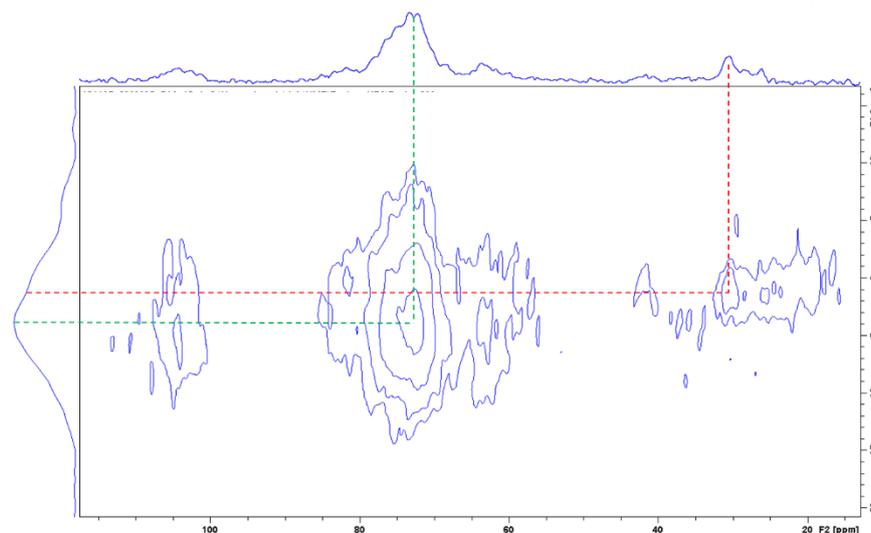
402

403　　**Figure 6 –** 2D WISE NMR spectrum recorded for the sample containing 100 wt. % Arabica coffee.

404　　The dotted lines indicate the main correlations identified in the plot.

405

406　　**4. Conclusions**

407　　Solid-state NMR spectroscopy with ^{1}H and ^{13}C as probe nuclei and employing a suite of distinct

408　　approaches (*e.g.*, CP and CP-SP methods for ^{13}C NMR; use of T_2 filter and selective measurements

409　　of T_1 for ^{1}H NMR; recording of 2D ^{1}H-^{13}C WISE spectra) has been shown in this work to be a

410　　powerful method for the characterization of roasted coffee samples at the molecular level. The use of

411　　these different approaches has allowed the detection of NMR signals originated from phases with

412　　marked distinct dynamics present in the heterogeneous structure of the material. The results were

413　　interpreted considering a scenario where lipids (high-mobility phase) and carbohydrates (rigid phase)

414　　contribute differently to the ^{1}H and ^{13}C NMR spectra. This scenario is thus consistent with the well-

20

known morphological characteristics of the coffee beans, containing well-separated oil-rich regions and carbohydrate-rich cell walls.

The potential of solid-state NMR spectroscopy to distinguish the contributions from Arabica and Robusta coffee variants in coffee blends was also demonstrated, illustrating the usefulness of this approach for screening and identification of counterfeit coffee products. As the ^{1}H NMR spectra (especially when recorded with use of the T_2 filter) are completely dominated by the lipid contributions and considering that the oil fraction is higher in the Arabica variety than in the Robusta one, specific ^{1}H NMR signals (e.g., associated with diterpenes) can effectively be used as indicators of the amount of Arabica coffee in a mixture. The differences detected in the spectral regions corresponding to the signals due to these compounds evidence a clear distinction between the Arabica and Robusta contributions. These differences can then be analyzed with the help of chemometric multivariate methods, which would allow the establishment of analytical methods to detect the amounts of Arabica and Robusta varieties in coffee blends based on the solid-state NMR approach; this is certainly a promising way to expand this line of investigation in future work.

The solid-state NMR experiments here described are non-destructive, relatively fast and straightforward to implement in commercial NMR spectrometers equipped with conventional solid-state probes. Moreover, the coffee samples are analysed as received, with no need to dilute or obtain lipophilic extracts (as required in solution NMR experiments), which also avoids problems related to sample degradation. It is clear that further studies are required to establish these methods as useful analytical tools for coffee screening purposes, such as the evaluation of the consequences of the variability in the oil contents of Arabica coffee samples from different regions and the establishment of the limit of detection of Robusta coffee fraudulently mixed with Arabica coffee. On the other hand, the straightforward sample preparation for solid-state NMR experiments, the reproducibility of the obtained results over time, the relatively short analysis time (*ca*. 20 min for ^{1}H and *ca*. 1.5 h for ^{13}C NMR experiments) and the promising correlations illustrated in this work stimulate the pursuing of

further in-depth investigations in this field, aiming applications of solid-state NMR as a practical method for the analysis of coffee products in the fields of food and forensic sciences.

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