STUDIES ON COFFEE ROASTING PROCESS BY MEANS OF NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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ABSTRACT

The chemical composition of coffee has been widely investigated, focusing the attention both on main components and trace compounds. Most of these studies have been performed by using liquid and gas chromatography, eventually combined with mass spectroscometry. These techniques, although straight and effective, are time demanding due to the sample pretreatments. Here, we propose high-resolution-magic-angle spinning nuclear magnetic resonance spectroscopy (HR-MAS NMR), a system capable of acquiring highly resolved NMR spectra of gel-like and suspension samples. This approach allowed us to determine the chemical composition of coarsely ground coffee beans of two varieties: Arabica and Robusta. Variation of the concentration of relevant species was monitored as a function of roasting temperature, from green beans to completely roasted. The HR-MAS NMR tool demonstrated to be very powerful for quick chemical composition determination, opening up possibilities for novel applications of this approach in food quality control.

PRACTICAL APPLICATIONS

Food quality control needs novel and press-button applications. High-resolution-magic-angle spinning nuclear magnetic resonance contains such requirements, since it can analyze suspension and gel-like samples, providing a chemical characterization with minor, or, in most cases, no chemical and physical pretreatment operations. The application proposed here concerns the examination of relevant molecules in coffee beans as a function of the roasting

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temperature. This can be used for a quality check of the final product, and such approach has a general suitability, being functional for almost all foodstuff, fresh and transformed.

INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy is an analytical approach widely used in food industry and food research. NMR can characterize the chemical composition of a foodstuff. It provides a very useful tool for the quality assessment by measuring quality-related attributes: identification of undesired substances, screening of chemical composition changes of complex mixtures, e.g., oils of different botanical origin (Fauhl *et al.* 2000), the detection of adulterating substances in orange juices (Vogels *et al.* 1996), etc. In the present study, we focus our attention on the evolution of relevant molecules as a function of roasting temperature in two of the most common coffee varieties: Arabica and Robusta. To reach such goal, we have used the innovative approach of high-resolution-magic-angle spinning (HR-MAS), a novel NMR probehead which allows us to obtain highly resolved spectra on gel-like or suspension samples. Such analytical system is particularly valuable since the prehandling operations of the sample are very limited. We have used it for the acquisition of ^{1}H-NMR spectra from manually and coarsely ground coffee beans. The first application of such HR-MAS tool in food science was presented in 2004 and took into consideration the chemical composition of Parmigiano-Reggiano cheese (Shintu *et al.* 2004). Afterwards, a few studies in food science have been reported in literature and extended this system to Emmentaler cheese (Shintu *et al.* 2004), beef meat (Shintu *et al.* 2004) and bread obtained by durum and tender wheat flour in Southern Italy (Brescia *et al.* 2007).

Coffee is a very common beverage among consumers, and there is an ongoing research for improving its quality; therefore an analytical tool for the quick and reliable chemical composition evaluation directly on coffee beans seems to be appealing.

Coffee beans contain hundreds of species (Clarke 1985), and a limited number is recognized to be responsible for the organoleptic and quality properties (Shibamoto 1991). Among these, sugars, aminoacids, acrylamide, pyrazines and melanoidines, which are all involved in the Maillard reaction occurring during roasting, may change their concentration largely.

Other relevant molecules that are important for the quality of the pleasantness of the final product, and undergoing chemical transformation during roasting, are trigonelline, N-methylpyrazine, chlorogenic acids, and caffeine (Kya *et al.* 2001; Campa *et al.* 2004). Trigonelline (3-carboxy-N-

methylpyridine) and chlorogenic acids (esters of caffeic or ferulic acid with quinic acid) are present in the green beans and are degraded during roasting. Caffeine is probably the most representative molecule in coffee; its concentration is not seriously affected by roasting, but is known to depend largely upon the variety.

A remarkable study on quality control and production reproducibility of a coffee mixture by means of NMR spectroscopy was carried out in 2002: statistical analysis on ^{1}H-NMR spectra of 98 coffee sample mixtures from three different producers was able to correctly identify the producer of a given coffee sample with a 99% probability (Charlton *et al.* 2002). Chemical characterization of espresso prepared by using different coffee varieties was as well achieved by NMR spectroscopy (Bosco *et al.* 1999). Furthermore, an NMR method for the investigation of the geographical origin of coffee was developed from the ^{13}C, ^{2}H, ^{17}O and ^{15}N isotopic distribution in caffeine molecule extracted from a coffee sample (Danho *et al.* 1992).

The aim of this study was to use the HR-MAS NMR approach for assessing the evolution of the concentration of significant molecules during the roasting process, especially referring to their role in the Maillard reaction, in Arabica and Robusta varieties. We consider HR-MAS a valuable system for investigating complex foodstuff mixtures, being capable of obtaining highly resolved NMR spectra of gel-like and suspension samples with very limited pretreatment operations.

MATERIALS AND METHODS

Sample Preparation

The varieties of coffee used were Arabica and Robusta; the green coffee beans were roasted in an aerated oven for 20 min at different temperatures: 30, 50, 70, 100, 140, 150, 160, 170, 180, 190, 200 and 215C, and then cooled down for 10 min at 4C. The roasted beans were manually and roughly ground, and a small amount, *c*. 5 mg, was inserted in a 4 mm Teflon rotor for HR-MAS NMR spectroscopy together with *c*. 40 mg of deuterated buffer at pH = 7.0, 0.01M (KH2PO4/K2HPO4) containing 0.5% TSP (tetra-silyl-propionic acid) used as internal standard.

NMR Spectroscopy. ^{1}H-NMR spectra were recorded at 25C with an AVANCE Bruker spectrometer (Bruker Biospin, Milan, Italy) operating at a proton frequency of 400.13 MHz. The sequence used contained the presaturation of the water signal (zgpr in Bruker library) obtained by centering the spectral window at 4.706 ppm and using a 2 s pulse with an attenuation of

64 dB. The spectral window (SW) was 11.015 ppm, the number of points of the spectrum were 32 K, and 90° proton pulses (4.50 μs and 5.30 dB attenuation) were used. Each spectrum was obtained with 1,024 scans, and the FID, prior to Fourier transformation, was multiplied by an exponential factor (line broadening) equal to 0.30 Hz. Phase and baseline corrections of spectra were manually performed by using the software package XWINNMR 3.5 (Bruker Biospin). Integration of the resonances of interest was obtained by using the same software package: relative integrals were normalized to the correspondent amount present in the 30C roasted sample, or to the internal standard TSP, in order to obtain the relative integral. Since two different references were used for normalization, a comparison between Arabica and Robusta with respect to the integral values (i.e., amount of each component) could not be made. The trends of each component of the two varieties versus temperature could be compared qualitatively: temperature of maximum amount, flat or steep profile of increase/decrease, were discussed. Statistical analysis was not performed since the number of replicas per sample was not significant, and despite this, it was not the aim of our study.

NMR signals were assigned according to data reported in literature (Bosco *et al.* 1999; Charlton *et al.* 2002), and were confirmed, being the sample a suspension and not in the liquid state, by means of standard 2D experiment. Little variation of δ, typically 0.02–0.05 ppm, was observed for few signals: pyrazines, N-methyl pyridine and chlorogenic acids.

RESULTS AND DISCUSSION

Coffee beans roasting process produces severe chemical modification through complex reactions. The Maillard reaction takes place and involves different classes of molecules: primarily sugars, amino acids, pyrazines and acrylamide. The reaction can be divided into three stages: in the first a Schiff base is formed by the reaction of the carbonyl moiety of a sugar with the amino group of an amino acid. The glycosylamine undergoes a rearrangement of the double bonds forming an Amadori (Molero-Vilchez and Wedzicha 1997) or Heyns compound, being the sugar an aldose or a ketose, respectively. Proteins, amino acids and sugars play a main role in the roasting process; asparagine reacts with glucose, giving N-D-glycosyl-L-asparagine, which is thought to be the precursor of acrylamide (Granby and Fagt 2004; Robert *et al.* 2004). In the second stage of the Maillard reaction, the Amadori-Heyns compounds are decomposed to form heterocyclic species, which are responsible for the coffee aroma (Czerny *et al.* 1999; Huang *et al.* 2007). More than 300 species have been identified in roasted coffee, including pyrroles, oxazols, furans, hymidazols and pyrazines (Clarke and Vitzthum 2001). The latter are present with

more than 80 compounds, 2-methylpyrazine being the largest in amount (Dart and Nursten 1985). Pyrazines arise from the oxidation of cysteine to cysteinsolfonic acid and cisteic acid (Little and O'Brien 1967), which do not contain thiol moieties, but sulfite groups, which are relatively inert. In these sulfur-lacking conditions, ammonia formed during the Maillard reaction is combined with pyrazine precursors and promotes their formation.

The third stage of Maillard reaction, through a still-unclear chemical mechanism, produces high molecular weight brown compounds of complex and unidentified structure, indicated as melanoidins (Nunes and Coimbra 2001). These compounds are probably formed via polymerization through aldolic condensation of intermediate unsatured carbons with hydroxymethylfurfurale; these polymers show side chains with amino groups (Kato and Tsuchida 1981; Cammerer and Kroh 1995; Yaylayan and Kaminsky 1998).

We analyzed the above described molecules by means of HR-MAS NMR; the acquired spectra were integrated to obtain an evaluation of the chemical composition of the samples.

Figure 1 shows the ^{1}H-NMR spectrum of Arabica coffee beans at different roasting temperatures, 30, 140, 180 and 215C from bottom to top. With increasing temperature, most of the peaks lose their fine structure as a consequence of polymers formation.

Sugars

The proton in position 1 of α-D-glucose shows a sharp doublet at *c.* 5.4 ppm, assigned from literature (Charlton *et al.* 2002), clearly visible in the NMR spectrum of the sample roasted at T = 30C. In both coffee varieties, we have observed (Fig. 2) a progressive reduction of α-D-glucose content, with a steep decrease, especially for Arabica, above 150C. This confirms that glucose is one of the reducing sugars involved in the Maillard reaction with amino acids (Oosterveld *et al.* 2003). The spectral region between 3.3 and 5.0 ppm contains the resonances of the other sugars present (Charlton *et al.* 2002). We have found very similar trends to the one observed for α-D-glucose for all analyzed sugars, especially fructose, sucrose, sorbitol, L-arabinose, galactose and mannose, for which the NMR signals are in the range 3.6–3.9 ppm (Bosco *et al.* 1999).

Amino Acids

These compounds, in particular asparagine and lysine, are relevant in Maillard reaction. Asparagine shows for the coffee sample roasted at 30C peak at $\delta = 4.01$ ppm, while the lysine one at $\delta = 3.52$ ppm (Charlton *et al.* 2002). Both signals arise from the proton in α-position with respect to carbonyl

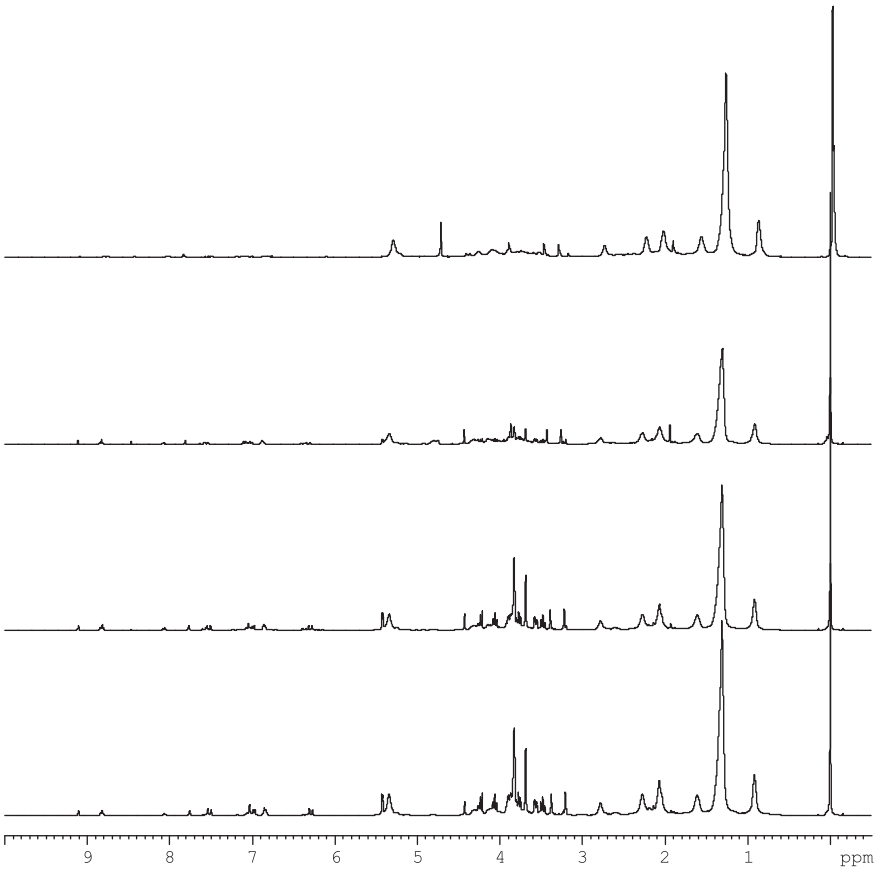

FIG. 1. [1]H HR-MAS NMR SPECTRA OF ARABICA COFFEE BEANS ROASTED AT DIFFERENT TEMPERATURES: 30, 140, 180 AND 215C FROM BOTTOM TO TOP, RESPECTIVELY

group. Their content decreases by increasing the roasting temperature (data not shown), following the same trend in both varieties and being very similar to those found for the sugars.

Acrylamide

Acrylamide, which is an intermediate product of the Maillard reaction, is formed from amino acids and has a relatively broad signal in the sample roasted at 30C at $\delta = 6.35$ ppm. We observed that for Robusta, its concentration reaches the maximum at 140C and then decreases. This trend, shown in

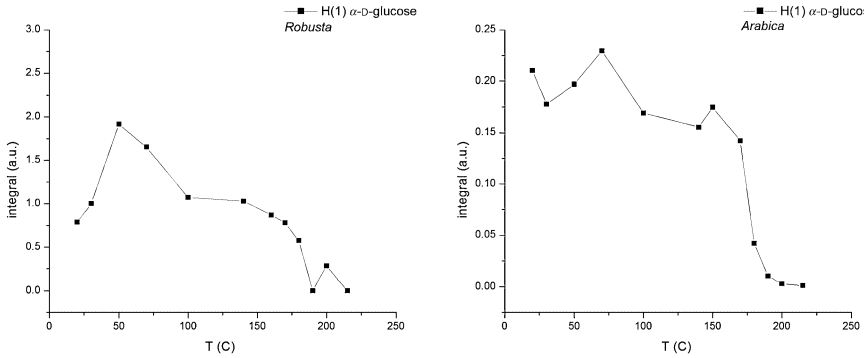

FIG. 2. RELATIVE VARIATION OF -D-GLUCOSE CONCENTRATION FOR ROBUSTA (LEFT PANEL) AND ARABICA (RIGHT PANEL) VARIETY VERSUS ROASTING TEMPERATURE

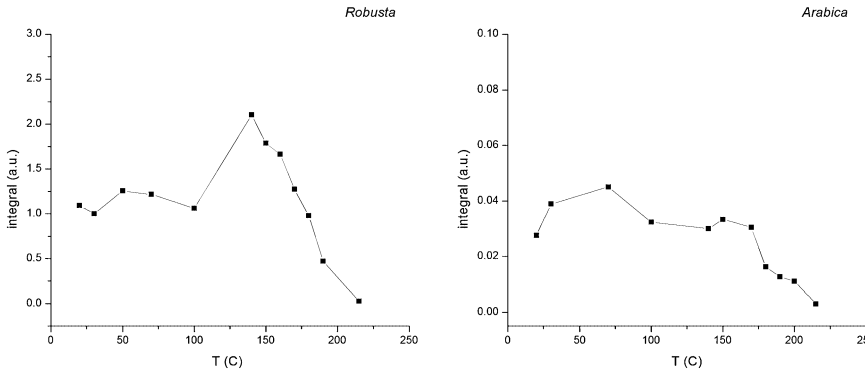

FIG. 3. RELATIVE VARIATION OF ACRYLAMIDE CONTENT IN ROBUSTA (LEFT PANEL) AND ARABICA (RIGHT PANEL) VARIETY VERSUS ROASTING TEMPERATURE

the left panel of Fig. 3, is in agreement with studies reported in literature (Gokmen and Senyuva 2006) and described by Eq. (1):

$$\frac{d[\text{acrylamide}]}{d\text{T}} = k_1[\text{asparagine}]^n \times [\text{sugar}]^m - k_2[\text{acrylamide}]^k \qquad (1)$$

In fact, from 30 to 140C, the reactants concentration is high, and acrylamide is produced; the rate is therefore positive due to the prevailing first term of Eq. (1). Above 140C, the acrylamide concentration decreases, and the rate, always referring to acrylamide, is negative, since the ruling term in Eq. (1) is the second. At T > 200C acrylamide is barely visible in the spectrum until com-

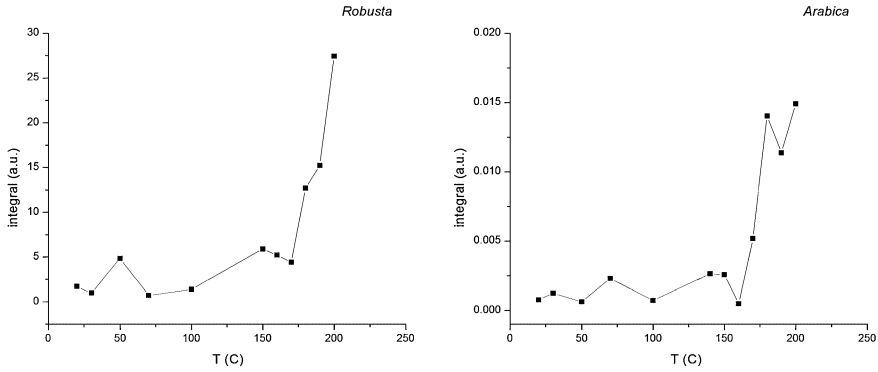

FIG. 4. RELATIVE VARIATION OF PYRAZINES CONCENTRATION FOR ROBUSTA (LEFT PANEL) AND ARABICA (RIGHT PANEL) VARIETY VERSUS ROASTING TEMPERATURE

pletely disappearing. The same trend was observed in Arabica variety, Fig. 3 right panel, where the highest acrylamide concentration was found at 70C. This might be due to the different values of the kinetic constants k_1 and k_2 in Eq. (1).

Pyrazines

These molecules, predominantly 2-methylpyrazine, are the main constituents of the coffee aroma (Hashim and Chaveron 1996). The singlet at *c*. 8.4 ppm (Bosco *et al.* 1999), arising from the proton in α-position with respect to the heteroatom of the aromatic cycle, becomes evident at temperatures above 140C, reaching the maximum intensity at the end of the roasting process, i.e., T = 215C.

Figure 4 reports the variation of the intensity of the NMR signal assigned to pyrazine, and one can observe that in both varieties, it is barely visible below 100C and increase smoothly until 150–160C, and then steeply above these temperatures. Their amounts change depending upon the roasting temperature, and not only upon the roasting time, as previously reported (Hashim and Chaveron 1996).

Melanoidins

Their presence determines the brown color of roasted coffee beans. The molecular structure is not yet completely established, and the correct assignment of the corresponding NMR signals is therefore very difficult. We have observed that spectral region between 3.4 and 4.6, Fig. 5, changed dramatically at the end of the roasting process, T = 215C, with respect to green coffee

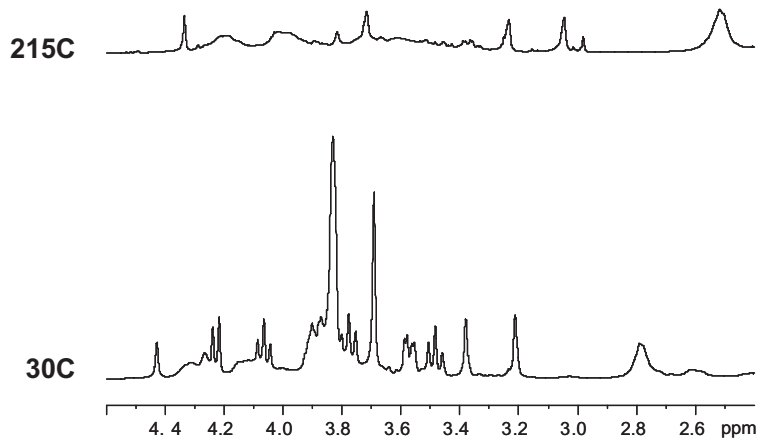

FIG. 5. EXPANSION OF THE UNASSIGNED MELANOIDINS SIGNALS REGION; FRESH COFFEE BEANS SPECTRUM (BOTTOM) AND COMPLETELY ROASTED ONE (TOP)

beans. This part of the proton spectrum contains the signals of melanoidins, and the broad and featureless peaks suggest the presence of compounds characterized by high molecular weight.

Trigonelline

The two resonances on the most left part of the spectrum are assigned to aromatic heterocyclic compounds, trigonelline above all (Bosco *et al.* 1999). In both coffee varieties considered, the signal at $\delta = 9.11$ ppm decreases already at 70C, approaching nearly zero when the roasting process reaches 215C (Fig. 6). Such reduction is more significant for Robusta (Fig. 6, left panel) because of higher starting concentration of trigonelline in the green beans.

N-Methyl-Pyridine

Trigonelline degradation gives as main products N-methyl-pyridine and nicotinic acid. The signal pattern of the latter was not assigned because of the pH, while a sharp triplet at *c*. 8.00 ppm corresponding to N-methyl-pyridine is clearly visible in the ^{1}H-NMR spectrum. N-methyl-pyridine resonance gains intensity during roasting, reaches a maximum and then goes to nearly zero, indicating a complete degradation of this molecule. The maximum concentration of N-methyl-pyridine occurs at 70C for Arabica variety, in accordance with the degradation temperature of trigonelline previously observed; while for Robusta variety, it is at 160C, indicating a major stability with respect to thermal degradation.

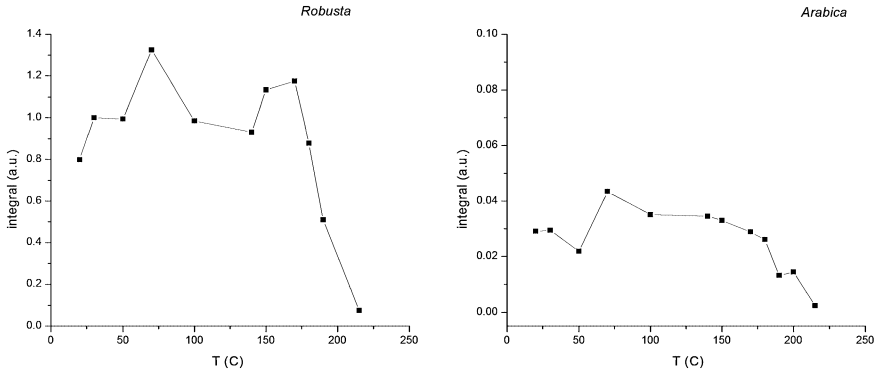

FIG. 6. RELATIVE VARIATION OF TRIGONELLINE CONCENTRATION FOR ROBUSTA (LEFT PANEL) AND ARABICA (RIGHT PANEL) VARIETY VERSUS ROASTING TEMPERATURE

Chlorogenic Acids

The presence of chlorogenic acids is evident in the ^{1}H-NMR spectrum by the sharp doublet at δc. 6.3 ppm (Bosco *et al.* 1999). The degradation curve as a function of the roasting temperature is very similar for both varieties: flat at low temperatures, steep at 140C for Robusta and at 70C for Arabica.

Caffeine

A signal attributed to caffeine was found at *c*. 7.8 ppm, as elucidated by literature (Bosco *et al.* 1999); its concentration is almost independent on roasting temperature. The interesting issue is the different concentration in Arabica and Robusta varieties; this is reported to be as one of the quality attributes that makes them different. We have calculated the concentration ratios of caffeine in Robusta with respect to Arabica for all the experimental temperatures. These data, reported in Fig. 7, show that the caffeine amount in Robusta variety is larger than Arabica, especially when the temperature becomes high. One of the main consequences of this observation is that Robusta is more acidic than Arabica, making the latter more pleasant and satisfying in taste.

CONCLUSIONS

The Maillard reaction is a browning nonenzymatic transformation, and plays a key role in the chemistry of thermally processed food. In the case of

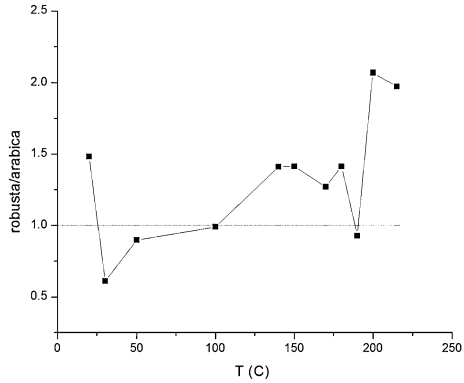

FIG. 7. CAFFEINE CONCENTRATION RATIOS BETWEEN ROBUSTA AND ARABICA VERSUS ROASTING TEMPERATURE

coffee beans, it produces color and aroma, primary quality attributes for the consumers. HR-MAS NMR spectroscopy has proven to be a valid analytical tool for a qualitative and quantitative investigation of species involved in the Maillard reaction, as well as other relevant molecules. HR-MAS approach is a powerful tool, as it does not require any prehandling treatment (e.g., extraction, purification, etc.) of the coffee beans, and generally speaking of any foodstuff. So that after, with very limited manipulation of the samples, we have monitored the variation of the concentration of sugars, amino acids, pyrazines, acrylamide, trigonelline, chlorogenic acids, pyridines and caffeine as a function of the roasting temperature, from 30 up to 215C.

This makes HR-MAS of potential interest for several applications in food science, both academic and industrial, with the clear advantage of avoiding time-demanding manipulation of the sample. The obtained NMR spectra are highly resolved and allowed a detailed chemical characterization of the species of interest. HR-MAS NMR, here applied to coffee beans, has a general validity, providing a quick tool for quality assessment.

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