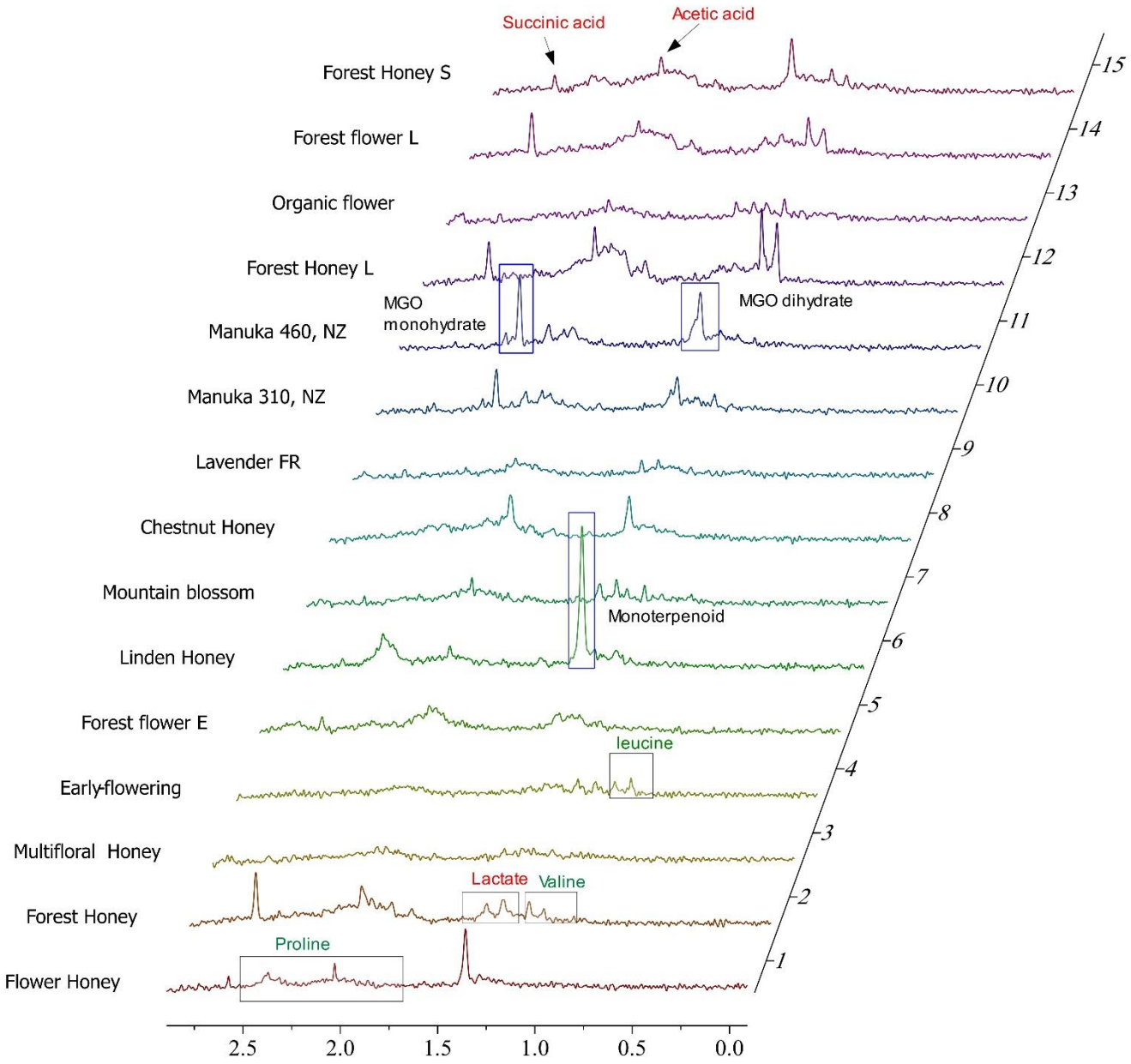
Honey Adulteration and Floral Origin Analysis by benchtop NMR spectroscopy



Natural honey is considered of high nutritional value and consumed every day in households and food industry. The market demand for honey far exceeds global production. The EU, for example, being the second largest honey producer in the world, is only approximately 60 % self-sufficient. This motivated honey fraud, such as mislabelling or adulteration with inexpensive sugar syrup. According to a recent report by the EU joint research center [1], 147 out of 320 honey (46%) imported from 20 countries were suspicious.

NMR is considered a useful tool for authenticating honey at chemical constituent level in a non-destructive manner [2]. The sample preparation and data acquisition time for NMR analysis are less demanding compared to other techniques. 1H-NMR allows immediate identification of sugars, amino acids, and other markers that are linked to adulteration and botanical origin. In the present study, we explore the potential of benchtop NMR spectrometer for honey analysis. A total of 26 commercial honey samples of different geographic and botanical origins were diluted directly in water (1:4, w/w) and analyzed on a Spinsolve 80 MHz Ultra system.

The moisture content in raw honey is up to 20%, and honey samples are usually diluted in buffer or water before NMR measurements. Therefore, solvent suppression is necessary to unveil the signals of interest under the large water signal. Figure 1 shows the superb performance of the WET sequence implemented on a Spinsolve 80 Ultra model. The narrow residual water peak is well resolved from the signals of the other constituents present in honey.

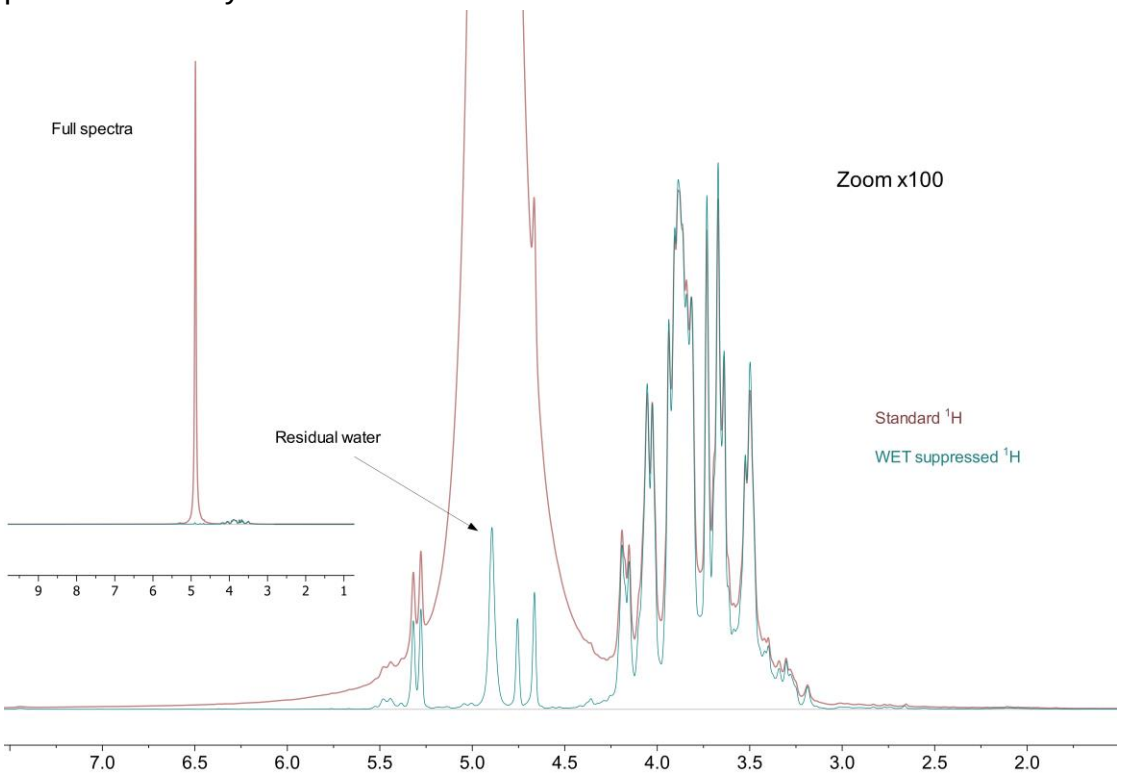


Figure 1: Standard 1H NMR spectrum of a honey sample superimposed with a spectrum of the same sample acquired with a WET solvent suppression sequence.

All 26 honey samples give a similar spectral profile after 2 scans (Figure 2), with all predominant signals from the two main monosaccharides, fructose and glucose. The sum of fructose and glucose should account for at least 60% in blossom honey [3].

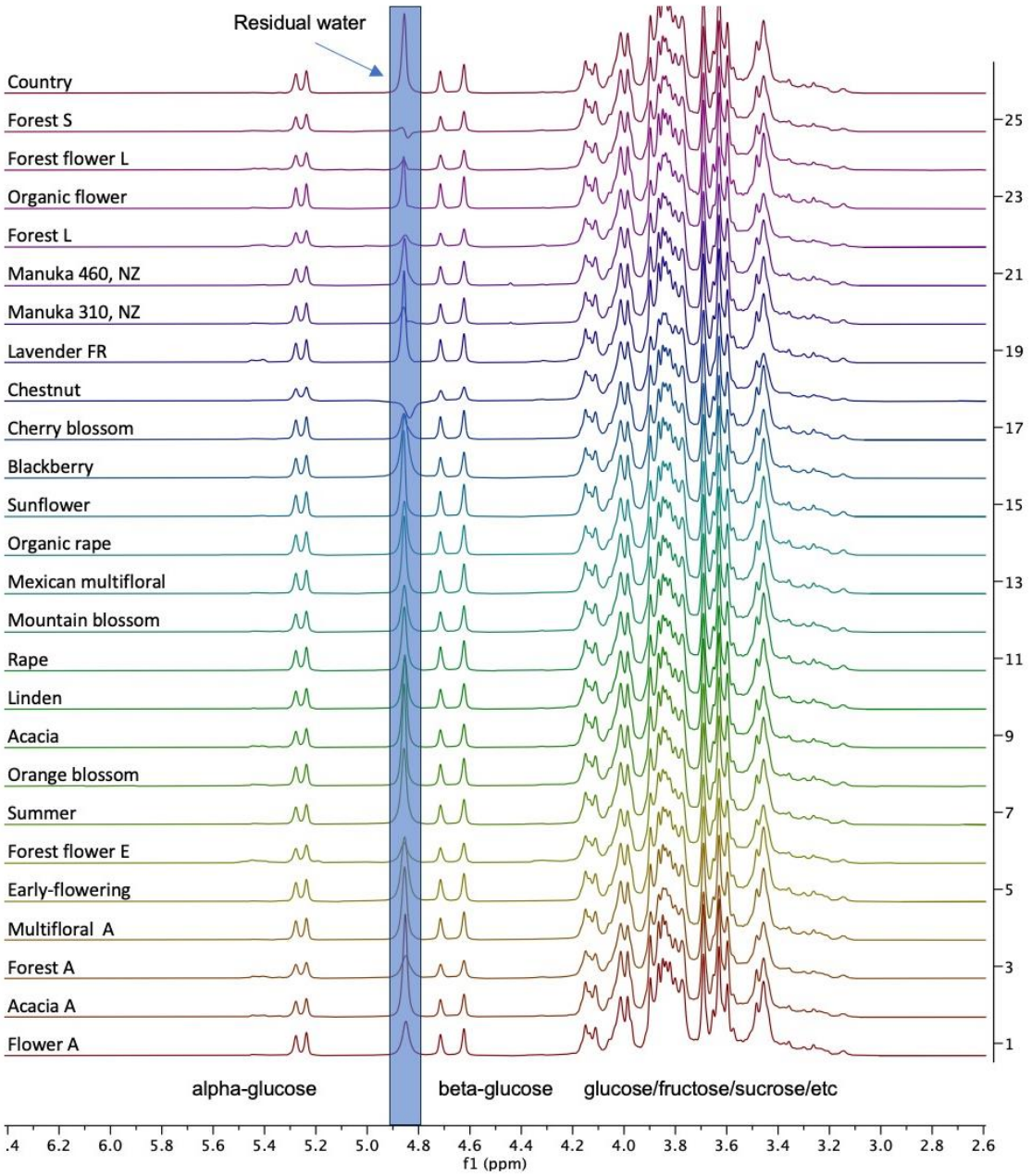


Figure 2: ^{1}H NMR spectra of 26 commercial honey samples from different geographic and botanical origins.

Longer measurement reveals other minor sugars in the anomeric region between 4.5 and 5.5 ppm (Figure 3). Literature values describe that the sucrose content, in general, should be under 5% in honey [3]. Turanose, an isomer of sucrose, is observed at about 5.3 ppm for all honey samples. This is a natural and inherent component of honey that can be used

as a marker to confirm authenticity [4]. On the other hand, mannose is a characteristic monosaccharide common in syrup and therefore serves as a particular marker for syrup adulteration in blossom honey [5].

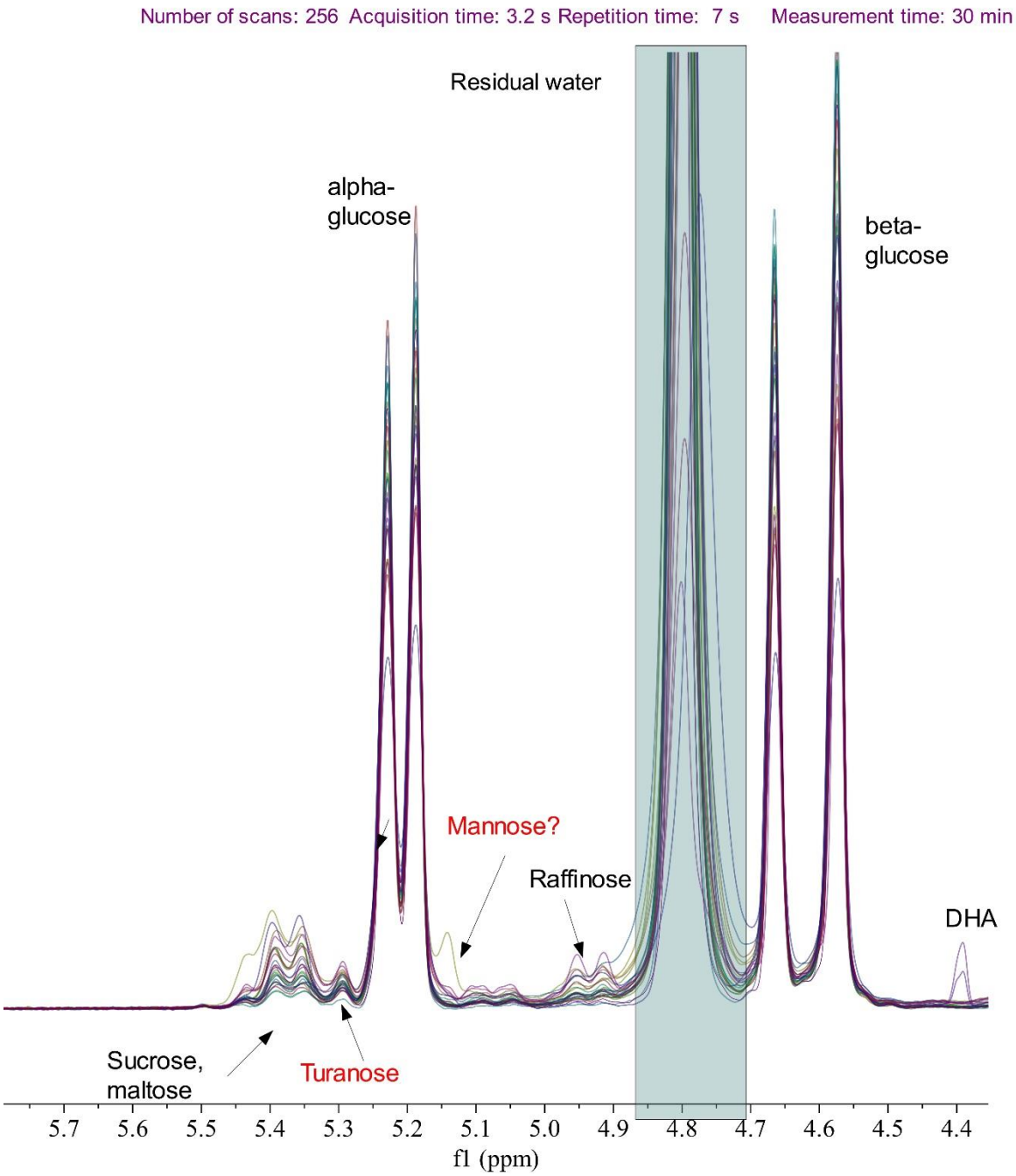


Figure 3: Zoom of the anomeric region of the ^{1}H NMR spectra shown in Fig. 2.

Zooming in the aliphatic region, the signal of some metabolites such as succinic acid, acetic acid, and lactic acid known to be produced during a fermentation process are visible in the aliphatic region. Proline as the main amino acid contributes to two broad signals between 2.0-2.5 ppm [6]. The two doublets at 1.10 and 0.91 ppm are from valine and leucine, respectively [7].

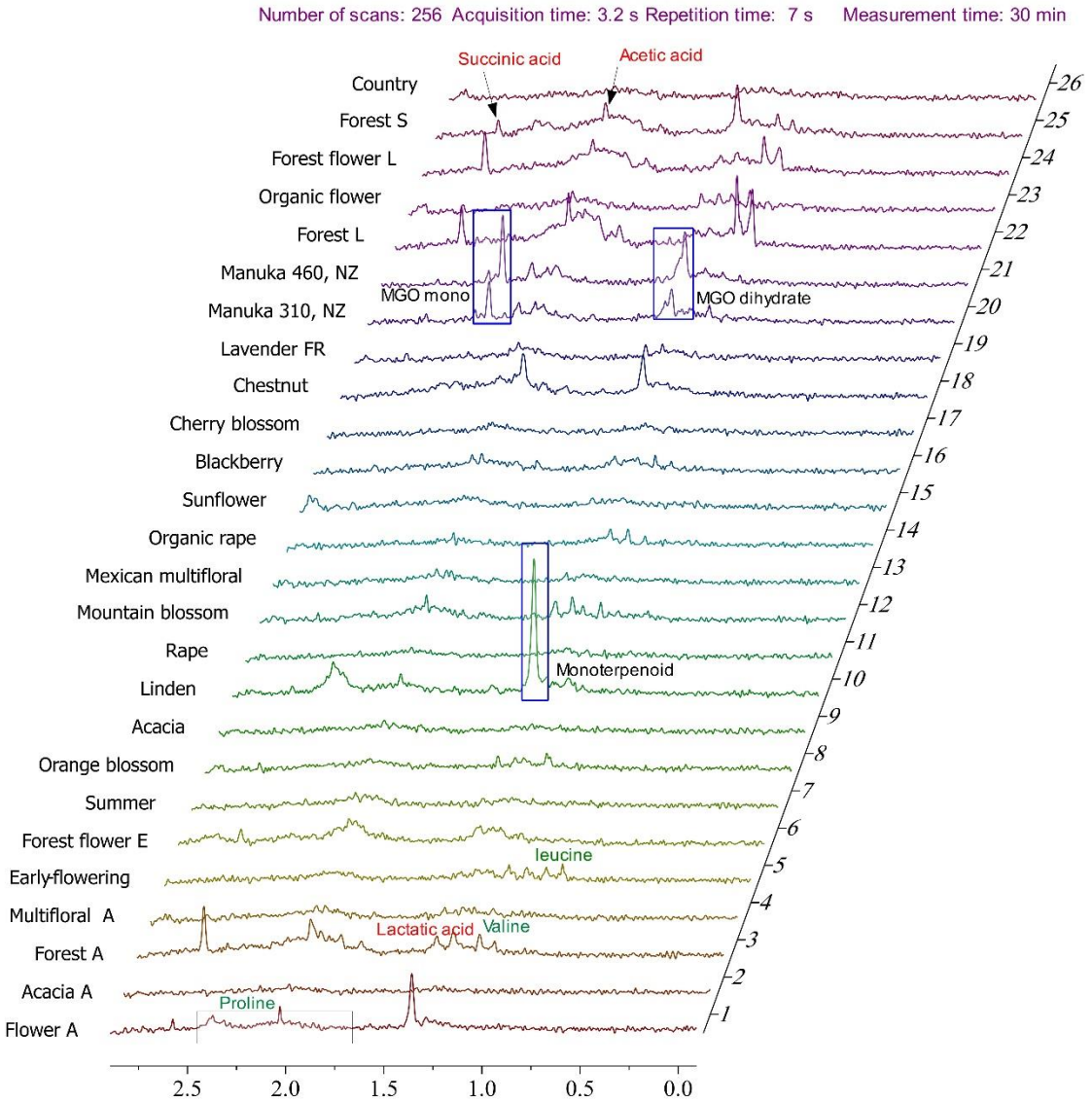


Figure 4: Aliphatic region of ^{1}H NMR spectra.

There are also components related to botanical origin. For example, the methylglyoxal (MGO) monohydrate at 2.27 ppm and dihydrate at 1.35 ppm (Figure 4), and the MGO precursor (Dihydroxyacetone, DHA, Figure 3) at 4.39 ppm are clearly visible in the two Manuka honey from New Zealand [8]. Linden honey contains much higher levels of monoterpenoid, cyclohexa-1,3-diene-1-carboxylic acid at 1.36 ppm, than any other honey [7]. On the other hand, in the aromatic region (Figure 5), the signals from tyrosine (7.2, 7.1, 6.9, 6.8 ppm) and high amount of phenylalanine at 7.36 ppm in lavender honey are also in good agreement with previous study [9]. Chestnut honey is characterized by kynurenic acid signals from 7.0 to 8.5 ppm [7]. The other compound that can be observed at 8.38 ppm corresponds to formic acid used for the treatment of varroa.

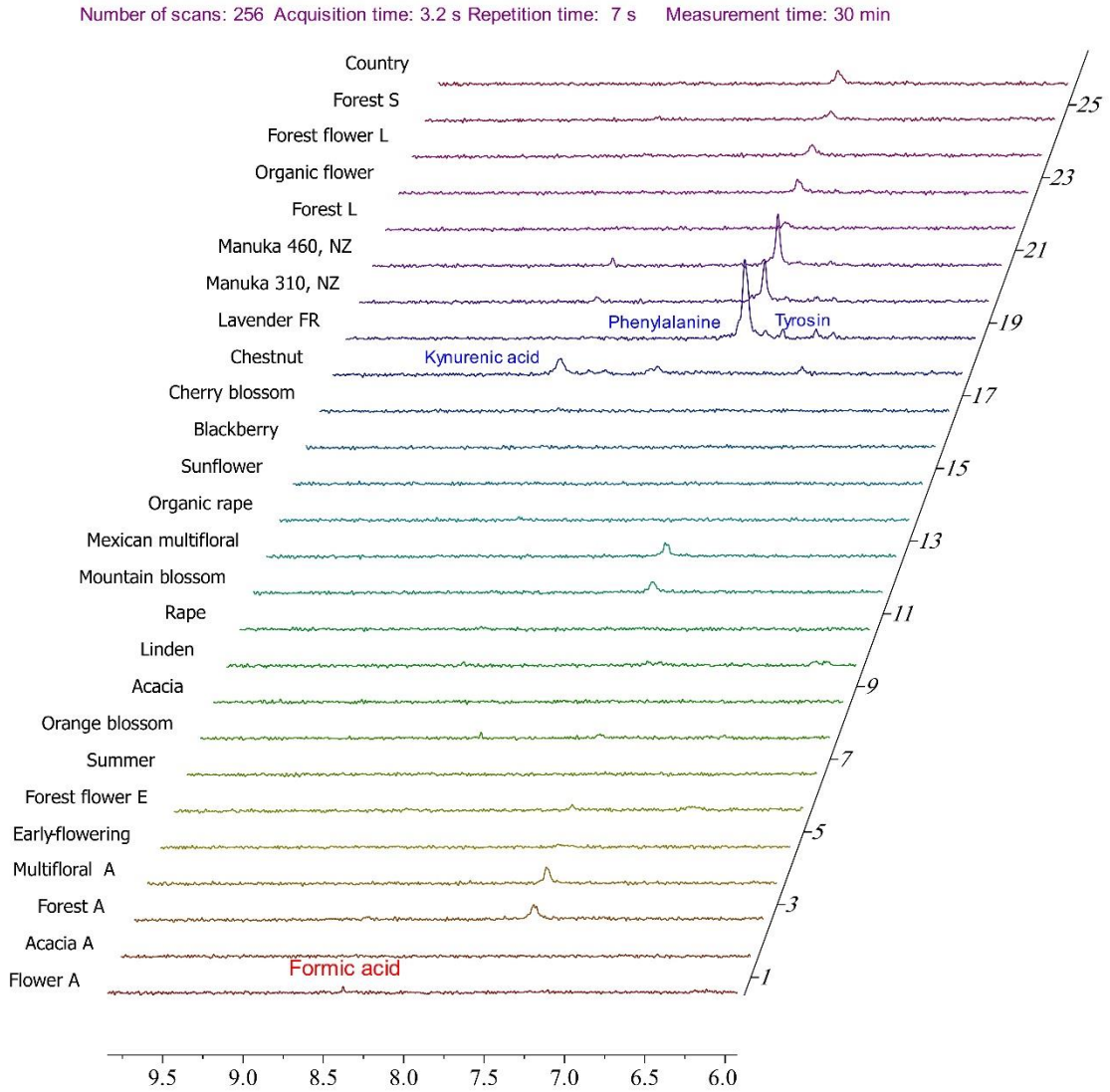


Figure 5: Aromatic region of ^{1}H NMR spectra

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